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EMBRYO ABORTION IN EARLY-RIPENING VARIETIES OF PRUNUS AVIUM

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(WITH FORTY-ONE FIGURES)

Introduction

The failure of early-ripening varieties of stone fruits, mainly of varieties of *Prunus*, to mature viable seed has been noted by both nurserymen and plant breeders. Nurserymen fail to secure good stands of seedling understocks of peach, mazzard cherry (*Prunus avium* L.), and mahaleb cherry (*P. mahaleb* L.) when the seed supply is obtained from wild trees which produce early-ripening fruit. Plant breeders find that crosses in which an early-ripening variety is used as the female parent produce viable seed only rarely.

BLAKE (1) pointed out that the flesh of some varieties of peaches ripens before the seed matures. MARSHALL (12) divided varieties of plum (*P. domestica* L.) into two groups, namely, those bearing mature fruits each of which contains a viable seed and those bearing mature fruits only a few of which contain viable seed. CHANDLER (3) reported never finding kernels in such early varieties of peach as the Sneed, although pollination is necessary for fruit production. CONNORS (4) stated that varieties of peaches ripening their fruit 75–80 days after pollination do not develop viable seed in New Jersey; that those which ripen 85–90 days after full bloom develop 15 per cent viable seed; and that later-ripening varieties produce a good

proportion of viable seeds. WELLINGTON (21) reported that records of cherry crosses indicated that early-ripening varieties used as parents had few viable seeds, medium-ripening sorts a higher percentage, and very late-ripening kinds a still higher percentage. TUKEY (18) showed that with 17 varieties and types of sweet cherry and 11 varieties and types of sour cherry, those kinds that were late-ripening (that is, requiring 80 days from full bloom to fruit ripening) developed nearly 100 per cent viable seed; those that were early-ripening (requiring 60 days or less from flower bloom to fruit ripening) developed almost no viable seed; and the mid-season sorts (those requiring 65–75 days to mature fruit) developed an intermediate number. SAKAGUCHI (17) recommends that plant breeders do not use as female parents peach varieties which ripen the fruit in fewer than 100 days after full bloom.

The problem presented by these facts suggests a critical comparison of the embryogeny and fruit development of early and late-ripening varieties of sweet cherry (*P. avium*). Furthermore, although critical studies have been made of both the early and the late stages in seed and fruit development, there is a dearth of material for the stages intervening. This paper attempts to supply information regarding these two phases of the subject.

Various phases in the embryogeny of the cherry have been reported by BRADBURY (2), MALPIGHI (11), PÉCHOUTRE (15), RUEHLE (16), TULASNE (20), WENT (22), and others. Studies in related genera which show similarity in development of related parts have been made by DORSEY (6) and OSTERWALDER (14). The growth of fruits of related species of *Prunus* has been studied by BLAKE (1), CONNORS (4), DORSEY and MCMUNN (7), LILLELAND (10), and SAKAGUCHI (17). A more detailed mention of these findings will be made with reference to the particular point involved.

Procedure

Fresh material was dissected under the binocular microscope. This method provided a rapid means of securing an adequate number of measurements of the various stages of development. In addition, it proved useful in helping to interpret the prepared slides.

Flower and fruit parts from twigs placed in water and held at

2°C. for 72 hours showed no measurable development, thus permitting the gross examination of a great number of individuals over a comparatively long period of time at identical stages of arrested development.

Material from open-pollinated blossoms in a mixed varietal orchard was most satisfactory for general study because of its more uniform rate of development. For comparison with the open-pollinated blossoms, however, blossoms were emasculated before they had opened and were pollinated by hand 3 days later, when the stigmas were receptive. Pollen was used which had been removed in the laboratory from closed blossoms and ripened at a temperature of 25°–30°C. Emasculated blossoms were kept covered with tight paper bags from the time of emasculation until the fruit had set. The paper bags were then replaced by cheese-cloth bags. Open-pollinated, self-pollinated, and cross-pollinated material was prepared, the crosses being between known compatible varieties and the pollen being tested by germination at room temperature (20°–25° C.) in 5 per cent sucrose solution.

Pollen tubes were traced in the carpel under the dissecting microscope from fresh material crushed in lacmoid-martius-yellow after the method described by NEBEL (**13**). The varieties used for critical study were Early Purple Guigne, Burbank, Lyons, and Black Tartarian, representing early-ripening kinds; Eagle, representing a mid-season sort; and Windsor, Lambert, Downer, and Mazzard, representing late-season varieties. Others examined for comparison were Governor Wood, Knight's Early Black, Elton, Napoleon, Schmidt, Black Republican, Abundance, and Oswego. The work was conducted at Geneva, New York, throughout the growing seasons of 1930, 1931, and 1932. Records for the years 1926 to 1929 inclusive have been used to corroborate the findings from the more exact studies of 1930 to 1932 inclusive.

Material for slides made after the paraffin method was killed and fixed in two solutions, designated solution I and solution II. Solution I was made up of 100 cc. of 50 per cent alcohol, 2.3 cc. glacial acetic acid, and 6.7 cc. formalin; solution II (Karpechenko's chromacetic) was made up of two parts, part A consisting of 1 per cent chromic acid in water and part B consisting of 10 cc. of glacial acetic

acid and 40 cc. of formalin. The two solutions were kept separate until used for fixation, when two parts of A by volume and one part of B were poured together. The material was first placed in solution I while being prepared, then placed in solution II, the whole being then exhausted to 600 mm. of mercury in a desiccator, and held at 4° C. for 24 hours. Excellent fixation was secured by this method, the material being equally as good for cytological as for histological studies. In the case of ovaries 6–12 mm. in length, longitudinal slices were cut from both sides of the ovary, parallel to the sutures and including portions of the nucellus, thus permitting rapid penetration. With larger material the seed was removed from the carpel.

Safranin, orange G, and crystal violet were used for staining.

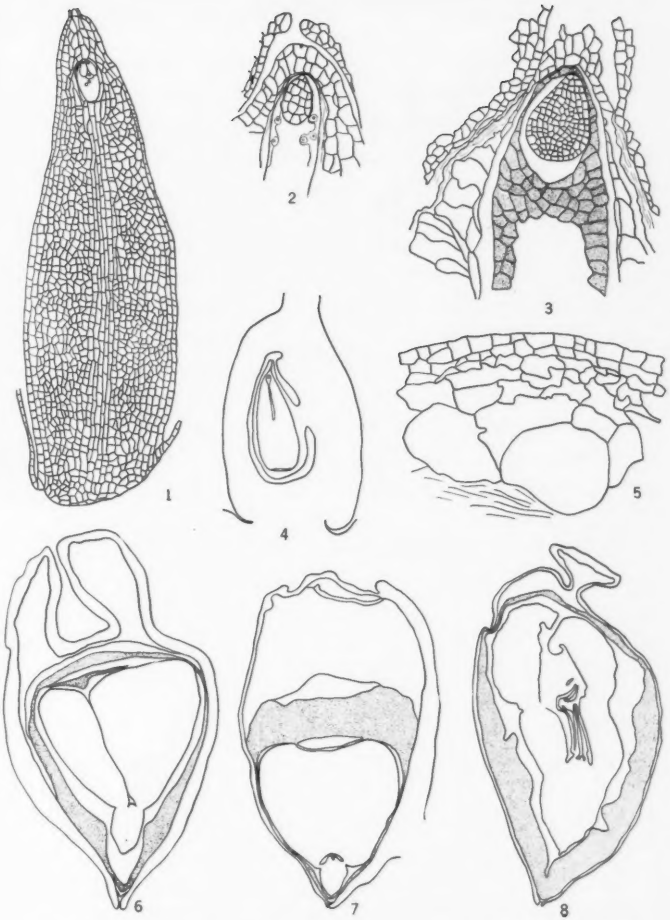
Measurements were made with an eyepiece micrometer, both sliding and fixed, and checked against each other. Care was necessary to secure median measurements and to secure fruits of average development. In the compilation of data shown in the tables, the mean of from three to seventeen individuals is represented for each measurement. The development of individuals collected at the same time showed very little variation, so that a smaller number would have served equally as well. Measurements from prepared slides were checked against those from fresh material dissected under the binocular microscope.

Normal development

Although certain stages and periods of development of particular parts of the cherry seed and fruit have been studied by other workers, no systematic record of development has been made, nor has any attempt been made to correlate the development of various parts. Accordingly the steps are here presented, and for this purpose the Downer variety of sweet cherry has been selected as typical or "normal." It is a late-ripening sort which normally produces a high proportion of viable seed.

1. OVARY.—The ovary increases in length only slightly from the 55th to the 14th day before full bloom, namely, from 0.54 mm. to 0.74 mm. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 2 mm. in length.

At the time of fertilization, a day or two after full bloom, there is



FIGS. 1–8.—Fig. 1, nucellus and 7-celled megagametophyte 2 days before full bloom, showing central core of conductive tissue of elongated cells from chalaza to megagametophyte through which the megagametophyte and embryo sac develop. Fig. 2, young embryo and free-nuclear endosperm 5 days after full bloom. Fig. 3, young embryo and cellular development of endosperm 12 days after full bloom. Fig. 4, development of megagametophyte, nucellus and integuments, and ovary from an emasculated, unpollinated blossom, 7 days after full bloom. Fig. 5, endosperm layer at chalazal end of seed, 37 days after bloom, showing growth by cell divisions in peripheral layer, region of cell enlargement, and region of digestion by adjacent advancing embryo. Figs. 6, 7, typical aborted seeds of Early Purple Guigne, 27 days after full bloom, showing peculiar flattening of cotyledons. Fig. 8, unusual embryo development in abortive seed, bearing 6 true leaves.

a sudden acceleration in the development of the pericarp, which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.46 mm. the 1st day after bloom to 3.31 mm. on the 4th, 4.38 mm. on the 6th, 7.5 mm. on the 13th, and to 10.5 mm. on the 17th. This is in contrast to the small increase prior to the time of fertilization. At the end of this period, development of the pericarp ceases almost as abruptly as it began, and a period of greatly lessened growth ensues, which in 1931 extended from the 17th to the 31st day, or a total of 14 days. A second period of rapid pericarp development follows the period of retarded development, continuing until the fruit is dead ripe, or from the 31st to the 66th day after full bloom during the season of 1931. Similar periods of accelerated and retarded pericarp development have been reported by BLAKE (1) and by DORSEY and MCMUNN (7) for the peach, and by LILLELAND (10) for the apricot (*Prunus armeniaca* L.).

2. NUCELLUS AND INTEGUMENTS.—There are two ovules, arising from opposite placentae of the single carpel, only one of which develops to maturity. The micropyle points toward the distal end of the ovary and the micropylar end of the ovule lies against an outgrowth of the placenta, the obturator (RUEHLE 16). Both ovules develop equally but slowly up to 14 days before full bloom, paralleling the development of the ovary. They then begin a more rapid enlargement and double in length by full bloom unless arrested in development.

At the central axis of the nucellus, reaching from the chalaza to the base of the megaspore mother cell or of the megagametophyte, is found a core of elongated cells as shown in figure 1, which take gentian violet stain readily and split apart readily in a longitudinal direction. The surrounding cells of the nucellus are more nearly isodiametric, thin-walled, with less dense cytoplasm. The chalazal region is clearly marked as a deeply staining tissue.

Two to 7 days before full bloom one of the ovules is abruptly arrested in development, as mentioned by RUEHLE, but does not shrivel and collapse until 3 or 4 days after full bloom. In some varieties, as Lyons, it is common for both ovules to develop until the period of embryo abortion.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached 5.71 mm. in size, compared with a maximum of 7.0 for Downer during the season of 1931. From this date until the fruit is nearly ripe there is a slight gain in size, and then a small decrease as the integuments shrink slightly at about the time of fruit ripening.

TULASNE (**20**) has remarked upon this early attainment of maximum size by the ovules of *Prunus spinosa* L., *P. avium*, and *P. mahaleb*, the ovule of the first reaching a length of 5 mm. before fertilization. PÉCHOUTRE, too, mentions that the ovule of *P. cerasus* L. reaches almost full size at a very early stage of fruit development.

3. MEGAGAMETOPHYTE AND EMBRYO SAC.—The stages in megaspore formation and megagametophyte development have been studied by RUEHLE (**16**) for the sweet cherry, by BRADBURY (**2**) for the sour cherry (*P. cerasus*), for several species of *Prunus* by PÉCHOUTRE (**15**), and by others. The development as carefully pictured and described by BRADBURY for the sour cherry seems identical with the development of the sweet cherry, so that a critical comparison would be superfluous. Accordingly only the time relationship will be emphasized.

The megaspore mother cell is first distinguished 14 days before full bloom, at the time the individual flower buds have separated in the cluster. It lies beneath five to eight rows of parietal cells, made up of the "coiffe épidermique" and the "calotte" of PÉCHOUTRE. It may remain in this stage for several days before reduction division. Following this division, disintegration of the three distal megaspores in the linear tetrad occurs rapidly. Thus the stages most commonly observed are either that of the megaspore mother cell or of the functional megaspore.

The megagametophyte is fully developed during the following 7 days, or 3 to 7 days before full bloom, at about the time the petals of the blossoms are showing. Development to the 7-celled stage is rapid. The polar nuclei, however, do not migrate to the center of the megagametophyte until about the time of full bloom, and then fuse a day or two prior to fertilization. A few starch grains are found in the megagametophyte at this time.

The synergids persist for some time, even 5 to 10 days after fer-

tilization. On the other hand, the antipodals disintegrate almost immediately after they are formed. The megagamete is relatively small and remains close to the micropylar end of the megagametophyte.

In the case of the non-functional ovule, which is commonly arrested in development just before full bloom, the megagametophyte may persist unelongated but otherwise complete for 10 to 14 days longer. Even after the nucellar tissue has collapsed, the integuments have shriveled, and degeneration has begun in these parts, the nuclei of the megagametophyte and of the megagamete may remain apparently unchanged, although arrested in growth.

At the time of fertilization there is a rapid elongation of the megagametophyte inclosed by the megaspore membrane. During the season of 1931 this increase in length was from 0.67 mm. 4 days after full bloom to 4.73 mm. 13 days later. It passes easily through the central core of elongated cells (fig. 1), as though in response to turgor pressure within, to assume a characteristically long tubular shape noted as long ago as 1675 by MALPIGHI (11), and called by him "vas umbilicale" and "umbilicus." Such acceleration is even more rapid than the growth of either the ovary or of the nucellus and integuments. It continues for a period of 17 days after full bloom, at which time the embryo sac, inclosed by the megaspore membrane, extends nearly to the chalaza. Development may begin 24 hours before pollen tubes can be found within the ovary, and therefore before fertilization has been accomplished; or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished.

This rapid development of the megagametophyte and embryo sac has been described by TULASNE for several species of *Prunus*, including *P. avium*, by PÉCHOUTRE who called it a "canal," and by WENT.

Occasionally the chalazal end swells to assume a balloon-like shape, and usually there is more or less narrowing about midway, similar to that described by WENT as an isthmus. WENT also pictures the antipodals as persistent at the chalazal end of the elongated embryo sac. This situation has not been observed in these studies. There is, however, a characteristically large endosperm nucleus

present at the tip of the elongating embryo sac at the chalazal end, which gives the embryo sac a pointed appearance.

Representation of the embryo sac by some investigators as a much-branched haustorial organ ramifying the nucellar tissue near the chalazal end, or as a much-enlarged balloon-shaped lacuna, is probably due to swelling and to artifacts occasionally produced in fixation. Such conditions are not found in fresh material.

The megaspore membrane, which is the outer layer of the embryo sac, is continuous and persistent. With dissecting needles it can be removed intact from fresh material, and still later in development the embryo, cellular endosperm, and embryo sac containing free-nuclear endosperm may all be removed together as though surrounded by the same continuous membrane. TULASNE (**20**) has also remarked upon the persistence of this membrane in dissection. The last vestiges of it may still be seen pressed against the chalazal portion of the nucellus by the developing endosperm 36 days after bloom.

The haustorial nature of the embryo sac will be discussed in connection with endosperm development.

4. FERTILIZATION.—Fertilization is as pictured for the sour cherry by BRADBURY (**2**). The sweet cherry, however, is self-sterile. In the case of emasculated flowers, hand-pollinated with tested pollen of known compatible varieties, pollen tubes had penetrated the style only slightly 24 hours after pollination. They were traced to the base of the style 48 hours after pollination in selfed flowers, but no further. In the case of cross-pollinated flowers, pollen tubes were found within the ovary, and occasionally within the micropyle at this time. In all open-pollinated blossoms, numerous pollen tubes could be found traversing the style, penetrating the ovary, and within the micropyle. The effect of emasculation and bagging is to retard the development of all parts from 4 to 6 days but otherwise to produce no unfavorable results.

5. ENDOSPERM.—Both the embryo and the endosperm develop very slowly for a considerable time after fertilization. The primary endosperm nucleus divides prior to the division of the zygote as described by BRADBURY for the sour cherry, and by PÉCHOUTRE.

It remains free-nuclear for some time (fig. 2). The nuclei of the chalazal region are large and relatively far apart. This portion never becomes multicellular. The nuclei of the micropylar region are smaller, close together, and this portion subsequently becomes cellular.

In *Pyrus*, the embryogeny of which is similar to that of *Prunus*, it has been shown by OSTERWALDER (**14**) that one of the first two endosperm nuclei migrates toward the micropylar end of the embryo sac and is the nucleus from which the cellular endosperm is subsequently derived; while from the other is formed the large-nuclear endosperm of the chalazal region. That this is probably the situation in *Prunus* is suggested by the material.

WENT has described the isthmus of constriction which divides the embryo sac midway into regions. PÉCHOUTRE discusses the elongate embryo sac containing free-nuclear endosperm from the standpoint of rapid movement of food materials from the chalazal region of the nucellus to the embryo and cellular endosperm in the micropylar end of the embryo sac. RUEHLE considers the embryo sac as a haustorial organ, and traces the movement of food materials from the vascular bundles of the integuments through the cells of the chalazal region and through the elongate embryo sac. OSTERWALDER considers the embryo sac of *Pyrus* as serving a haustorial purpose. He suggests that the difference in the shape and size of the endosperm nuclei at opposite ends of the embryo sac is due to nutritive conditions. He also suggests that cell walls are not laid down beyond the isthmus toward the chalazal region because of the chemical make-up and nutritive function of that region.

Cell walls begin to appear the 9th day after full bloom between the nuclei surrounding the embryo at the micropylar end of the embryo sac. Cell wall formation subsequently continues slowly until the 17th day after full bloom, the cellular endosperm reaching a length of only 0.57 mm. at this time during the season of 1931. The cell walls are formed progressively from the micropylar to the chalazal end, and from the periphery inward, much like the deposition of layers of material upon the inside of a pear-shaped bag until it becomes a solid mass (fig. 3). At the region of the isthmus reported by WENT cell walls are sometimes formed, and then often with sev-

eral nuclei in one cell. In the region of the chalaza no cell walls are formed. The increase of the endosperm after it has formed this cellular mass is still from the micropylar toward the chalazal end, but is by cell division rather than by the addition of cell walls to free-nuclear endosperm.

This period of delayed development is followed by a period of very rapid enlargement in which the cellular endosperm increases from 0.57 to 4.8 mm. between the 17th and 31st day after full bloom. There is an active region of cell division at the periphery of the cellular mass of endosperm (fig. 5) away from the micropylar end. The newly formed cells enlarge and are in turn digested by the advancing embryo. There is no indication of the endosperm being "pushed ahead" of the developing embryo, as has been stated in the literature. In the thinner portions of the endosperm it may be only 6–8 layers of cells thick. At maturity, the seed is entirely surrounded by a thin layer of endosperm 0.15 mm. thick at the chalazal end.

6. EMBRYO.—The first division of the zygote is to form a large suspensor cell. The suspensor is small, as described by PÉCHOUTRE and RUEHLE, and similar to that described for the sour cherry by BRADBURY. It is persistent to maturity of the embryo. The development of the embryo is even slower than that of the endosperm. Four days after full bloom in 1931 the embryo had passed the zygote stage, but from then until the 24th day after full bloom it had reached a total length of only 0.16 mm. In the early stages of embryo development, the embryo sac contains many tiny starch grains as reported by BRADBURY.

At this time there occurs a sudden rapid development so that the embryo increases in length from 0.71 mm. on the 27th day after full bloom up to 3.00 mm. on the 31st day. Material killed and fixed during this period shows numerous mitotic figures of various stages throughout the embryo. Development from this point is regular but less rapid. The end result is an embryo with two well developed cotyledons, surrounded by a thin layer of endosperm and a fine line of nucellar tissue, the whole completely filling the integuments.

DORSEY and MCMUNN have noted a similar slow, early development of the embryo in the peach (*Prunus persica*), followed by very rapid enlargement. They give the length of the young embryo in the

Elberta peach as only 1/16 of an inch two months after full bloom, and then describe a period of rapid development for one month.

7. EMBRYO ABORTION.—When embryo abortion occurs it is observed as a sudden arrest in development of the embryo. The nucel-

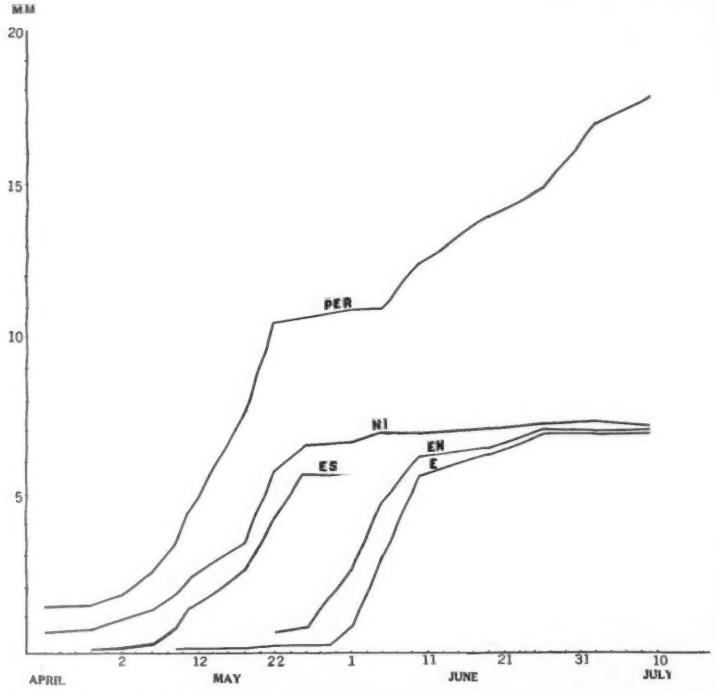


FIG. 9.—Graph of typical development of pericarp (*per*), nucellus and integuments (*ni*), megagametophyte and embryo sac (*es*), endosperm (*en*), and embryo (*e*) of a late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed.

lar tissue collapses, the integuments shrivel, and the characteristic aborted seed shown in figures 6 and 7 is the result. The aborted embryo may begin to disintegrate 5 days after arrest in development, or it may remain intact for at least 42 days. There is great variation in the stage of development of the fruit and in the stage of development of the embryo at which abortion occurs.

Critical examination of embryos of dropped fruits from the sweet cherry variety normally producing viable seed has shown no apparent structural variation from a developing embryo of a developing fruit. Neither does there appear to be any structural variation in the

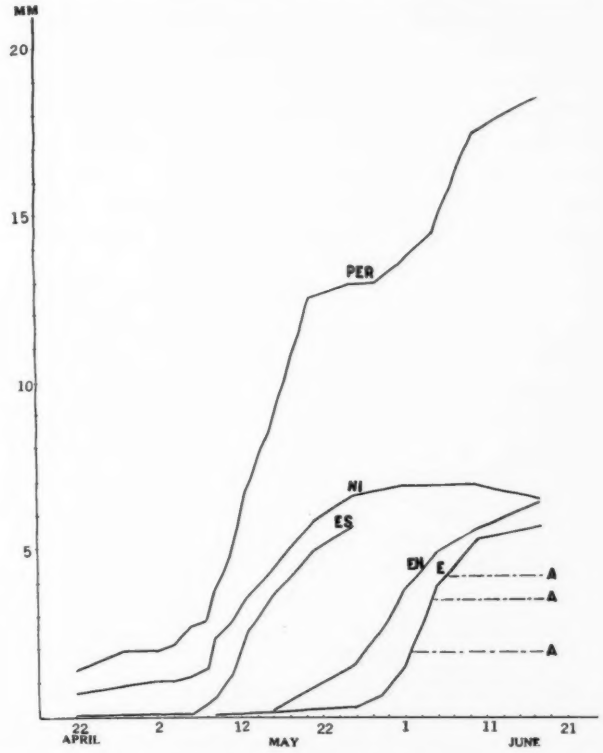


FIG. 10.—Graph of typical development of pericarp, nucellus and integuments, megagametophyte and embryo sac, endosperm, and embryo of an early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed.

nucellus or the endosperm of ovules containing abortive seed, with the exception of a suggestion of collapse of nucellar tissue adjacent to the developing cellular endosperm. On the other hand, there is a tendency for the embryo in dropped fruits to continue development,

as reported by BRADBURY. Figure 8 shows an unusual development of an abortive embryo in a dropped fruit. The embryo has "germinated" and has developed six true leaves.

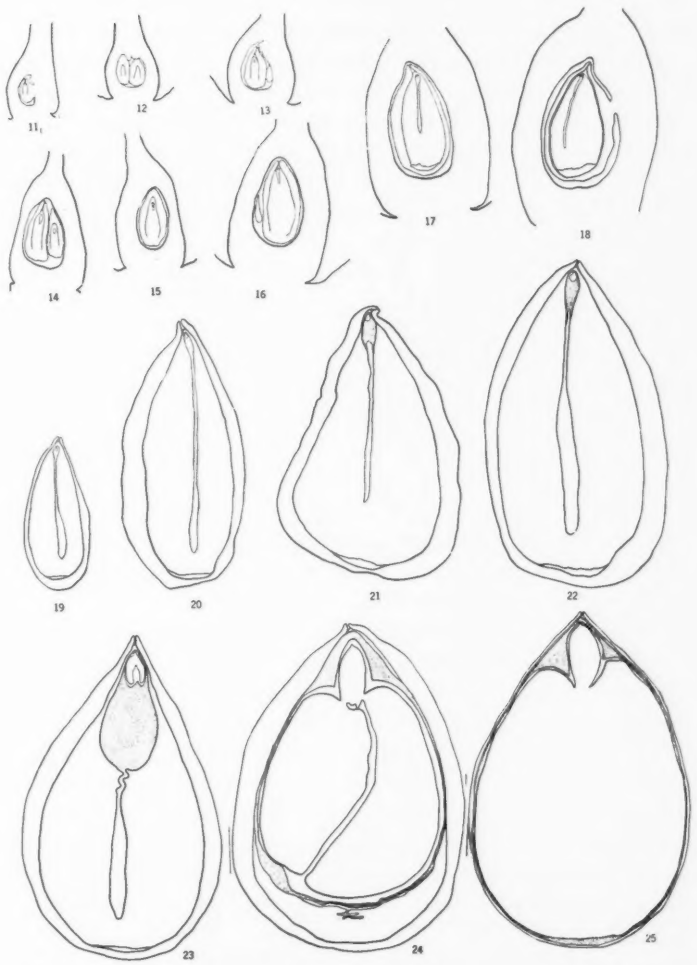
Usually embryo abortion is associated with dropping of the fruit. BRADBURY has shown the various waves of fruit drop for the Montmorency sour cherry and has explained them as resulting from factors of nutrition. KRAUS (**8**) has pointed out that most fallen fruits contain embryos in some stage of development. DETJEN (**5**), working with the apple, pear, and plum, has reported that most of the fruits of the June-drop stage contain embryos or tissues resulting from fertilization. He also points out, however, that there is a varietal character involved in the dropping of fruits; that is, trees of the same variety under identical conditions react similarly whereas trees of unlike varieties react differently.

On the other hand, embryo abortion may also be a characteristic of mature fruits of certain varieties. Mention has been made earlier in this paper of the findings in this regard of MARSHALL (**12**) for the plum; BLAKE (**1**), CHANDLER (**3**), CONNORS (**4**), and SAKAGUCHI (**17**) for the peach; and WELLINGTON (**21**) and TUKEY (**18**) for the cherry.

8. CORRELATION OF DEVELOPMENT.—The development of the various parts discussed in the preceding pages in relation to one another is shown graphically in figure 9. Figures 11 to 25 present camera lucida drawings of development at given stages.

In brief, the ovary and ovule parallel each other in development during the early stages. They develop only slightly from the 55th to the 14th day before full bloom. Between then and the time of full bloom the ovary and the nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3–7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the ascendency.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte and embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time the development of the ovary is abruptly arrested. The nucellus and



FIGS. 11–25.—Camera lucida drawings of stages in development of typical late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed. Days before full bloom: fig. 11, 13 days; fig. 12, 7 days; fig. 13, 3 days. At full bloom: fig. 14. Days after full bloom: fig. 15, 1 day; fig. 16, 4 days; fig. 17, 6 days; fig. 18, 8 days; fig. 19, 13 days; fig. 20, 17 days; fig. 21, 18 days; fig. 22, 21 days; fig. 23, 27 days; fig. 24, 36 days; fig. 25, 67 days.

integuments also slow down in growth, but not so sharply. The endosperm and embryo make no appreciable growth.

During the period of retarded development of the pericarp, namely, from the 17th to the 31st day after full bloom, there is a sudden acceleration in growth of the endosperm and embryo. A second period of rapid pericarp development follows the period of retarded development, extending from the 31st day after full bloom to fruit ripening. At this time the nucellus and integuments reach maximum size. The endosperm and embryo continue their development, although at a slower rate. The end result is a ripe fruit containing a viable seed.

Development of an early-ripening variety

For the purpose of tracing the development of an early-ripening sweet cherry, the Early Purple Guigne was selected. This variety is typical of early-ripening sorts which normally produce few or no viable seeds.

1. OVARY.—The ovary increases in length only slightly from the 40th to the 14th day before full bloom. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 1.94 mm. long.

At the time of fertilization there is a sudden and very rapid acceleration in development of the pericarp which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.01 mm. the day before full bloom to 2.68 mm. the day following. It measured 3.80 mm. the 5th day after bloom, 4.87 mm. on the 7th, 6.75 mm. on the 9th, 8.5 mm. on the 12th, and 12.5 mm. on the 17th. At the end of this period, development of the pericarp ceases almost as abruptly as it began and a period of greatly lessened growth ensues, which in 1931 extended over a period of 8 days, from the 17th to the 25th day following full bloom. The stony pericarp was becoming brittle at the end of this period, and the fruit was beginning to color.

A second period of rapid pericarp development follows the period of retarded development, commencing on the 25th day after full bloom and continuing until the fruit is ripe, namely, the 37th day after full bloom during the season of 1931.

2. NUCELLUS AND INTEGUMENTS.—Both ovules develop equally but slowly up to 14 days before full bloom, after which there is a more rapid enlargement, and they double in length by full bloom, unless arrested in development.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached a length of 5.85 mm. compared with a maximum of 6.96 mm., reached 26 days after full bloom. At various stages, beginning at this time, the nucellar tissue suddenly collapses and the integuments shrivel and collapse. There is no apparent structural abnormality prior to this collapse, with the possible exception of the region of contact between the nucellus and the developing cellular endosperm. At this point there is a loss of turgor in the nucellus just prior to the general collapse of the nucellus and integuments. The phenomenon suggests a sudden decrease in turgor in the thin-walled cells of the nucellar tissue. In this variety seeds are rarely found with integuments which have not collapsed.

3. MEGAGAMETOPHYTE AND EMBRYO SAC.—Development of the megagametophyte and embryo sac is similar in every way to that of the normal variety already described. It progresses regularly and uniformly, no abnormalities being disclosed by a critical study.

4. FERTILIZATION.—Pollination and fertilization are as outlined for the variety already described. Pollen tubes were traced through the style, within the ovary and within the micropyle, in the case of both open-pollinated and also emasculated flowers pollinated by hand. Tested pollen was used of known compatible varieties, as already discussed.

5. ENDOSPERM.—Development of the endosperm following fertilization progresses similarly to that already described. It is free-nuclear until the 9th day after full bloom, at which time the first cell walls appear. Twelve days after full bloom the cellular endosperm has reached a length of only 0.17 mm. By the 22nd day after full bloom, however, a period of rapid acceleration has begun, during which the cellular endosperm reaches a length of 3.73 mm. 28 days after full bloom, and 5.6 mm. 37 days after full bloom.

At various stages from the 28th day after full bloom until fruit ripening, there occurs a sudden arrest in development of endosperm

formation. Structurally there is no apparent change prior to the time of suspended development.

6. EMBRYO.—The embryo also develops similarly to the previous variety. It develops very slowly following the first division of the zygote, until by the 22nd day after full bloom it has reached a length of only 0.25 mm. At this time it begins a sudden and rapid enlargement, so that in some individuals a length of 1.48 mm. has been reached 28 days after full bloom and 4.42 mm. 32 days after full bloom.

Throughout this period of rapid enlargement, however, there is a sudden cessation of growth in various individuals beginning 25 days after bloom, when the embryos are 0.52 mm. in length. Embryos may be arrested in development at any stage (figs. 6, 7). Structurally there is no apparent change in the embryo prior to abortion. The embryo seems merely to be checked in development during its period of most rapid growth. A similar situation has been reported by DORSEY (6) for the plum.

The aborted embryos often develop a peculiar shape, however, as shown in figures 6 and 7. They are often flattened at the tips of the cotyledons, and one tip is frequently curled over the other. It is suggestive of the flattening of the cotyledons against the arrested endosperm and the collapsed nucellus and integuments, as though the embryo had continued its growth for a period after the initial check in development. BRADBURY reported that the embryos of dropped cherries continued their development for some time later. It must be remembered, however, that she was dealing with aborted embryos in dropped fruits, whereas these results deal with aborted embryos in developing fruits.

There is a general grouping of aborted embryos into four waves, not unlike the waves of dropping fruits reported by BRADBURY. One occurs when the embryo has reached a length of approximately 2.9 mm., another when a group of embryos has reached 3.64 mm., again at a stage of 4.20 mm., and again at 5.73 mm. These groups are not absolute, but mark the points of greatest frequency. The writer has found no viable seed from this variety from several thousands that have been examined over a period of several years. The aborted embryos may begin to disintegrate within 5 days, or they may continue in a viable condition for at least 42 days.

7. Correlation of development.—Development of the various parts in relation to one another is shown graphically in figure 10. In addition, figures 26 to 40 present development at given stages as camera lucida drawings.

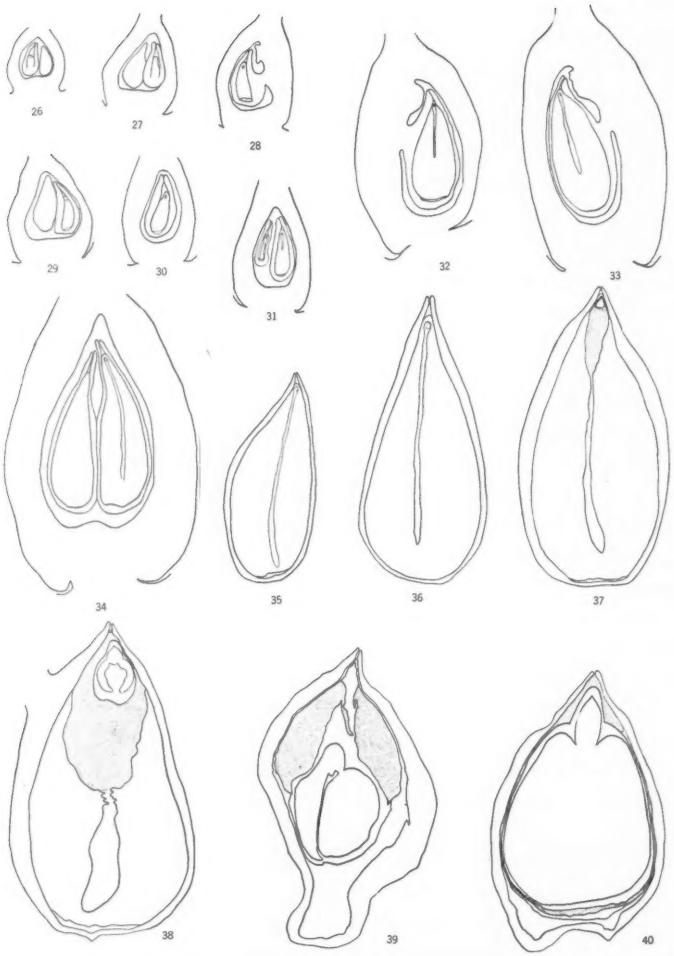
Briefly stated, the ovary and ovule parallel each other in development in the early stages. They increase only slightly in size between the 40th and 14th day prior to full bloom. Between then and the time of full bloom the ovary and nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3 to 7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the lead.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time pericarp development is abruptly arrested. The nucellus and integuments also slow down in growth but not so sharply. The endosperm and embryo make no appreciable growth. During the period of retarded pericarp development, beginning on the 22nd day after full bloom, there is a sudden acceleration in growth of the endosperm and of the embryo.

The second period of rapid pericarp development, which follows the period of retarded development, extends from the 25th day after full bloom until the fruit is ripe on the 37th day. Thus the second period of rapid pericarp development is initiated and the fruit is beginning to color at the time the embryo and endosperm are in their period of most rapid development.

The nucellus and integuments reach maximum size on the 25th day after full bloom. Beginning at this time, and throughout this second period of rapid pericarp development, there is a sudden cessation of both endosperm and embryo development at various stages of development in various individuals.

The pericarp continues its development to the ripening of the fruit. The embryos abort at various stages, and the nucellus and integuments collapse. The end result is a ripe fruit containing a seed with an aborted embryo.



FIGS. 26–40.—Camera lucida drawings of stages in development of typical early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed. Days before full bloom: fig. 26, 12 days; fig. 27, 6 days; fig. 28, 2 days. At full bloom: fig. 29. Days after full bloom: fig. 30, 2 days; fig. 31, 4 days; fig. 32, 5 days; fig. 33, 7 days; fig. 34, 9 days; fig. 35, 12 days; fig. 36, 17 days; fig. 37, 22 days; fig. 38, 28 days; fig. 39, 32 days; fig. 40, 37 days.

Comparative development of early and late-ripening varieties

Dividing development into the stages of (1) pre-bloom, (2) pollination and fertilization, (3) first rapid pericarp development, (4) retarded pericarp development, and (5) second rapid pericarp development, the comparison between the early and late-ripening types may be summarized as follows.

During the pre-bloom period, both kinds of varieties develop similarly in all parts. Pollination and fertilization are likewise similar, except that the early-ripening variety develops slightly in advance of and at a slightly greater rate than the late-ripening variety.

During the period of first rapid pericarp development, beginning at the time of fertilization and continuing until 17 days after full bloom, there is also close similarity. During the period of retarded pericarp development the first significant difference between the two types is reached. In the case of the early-ripening variety, this period extends from the 17th to the 25th day after full bloom, or a span of 8 days. In the case of the late-ripening variety the period extends from the 17th until the 31st day, a span of 14 days. The period of rapid embryo and endosperm development begins about the same time for both types, namely, 25 days after bloom.

The period of rapid pericarp development in the early-ripening variety begins at the time rapid embryo and endosperm development are initiated; in the late-ripening variety this period does not begin until 6 days later. That is to say, at the time the second period of rapid pericarp development begins in the early-ripening variety, the cellular endosperm has reached a length of 2.40 mm. and the embryo a length of 0.52 mm. At the time this period begins for the late-ripening variety the cellular endosperm is 4.8 mm. in length and the embryo 3.0 mm.

Furthermore, the increase in pericarp development in the late-ripening variety continues at a much slower rate. It increases 2.6 mm. in 12 days as contrasted with an increase of 4.5 mm. for the first 12 days of this period for the early-ripening variety. Development of the late-ripening variety continues over a longer period of time, until 66 days from full bloom as contrasted with 37 days for the early-ripening variety.

Embryo and endosperm development of the late-ripening variety

is thereafter regular and uniform until a viable seed is produced. During this period embryo and endosperm development of the early-ripening variety is suddenly checked at varying stages of development, and a viable seed is produced only rarely. The nucellus and integuments collapse to give the characteristic shriveled seed of the early-ripening variety as contrasted with the plump, well-filled seed of the late-ripening type.

Results with other varieties

That the situation described for Early Purple Guigne, representing an early-ripening variety, and for Downer, representing a late-ripening kind, is characteristic also of other varieties is shown in table I, taken from a previous publication (**18**), showing the general relation between time of fruit ripening and viability of seed.

TABLE I

RELATION BETWEEN TIME OF RIPENING OF FRUIT AND VIABILITY OF SEED OF SWEET CHERRY IN 1927

VARIETY	NO. OF SEEDS	DAYS BETWEEN BLOOMING AND RIPENING	PERCENTAGE VIABLE SEED
Abundance	1915	82	94.5
Black Republican	3320	80	91.0
Black Tartarian	2437	62	7.2
Downer	8897	70	98.3
Eagle	1850	64	61.9
Early Purple	2494	48	00.1
Elton	2025	60	00.0
Governor Wood	2275	53	7.2
Knight	1600	56	00.0
Lambert	2220	78	55.0
Mazzard (Geneva)	4420	86	100.0
Mazzard (Virginia)	454	Over 80	97.5
Mazzard (Virginia)	624	Over 80	93.2
Napoleon	1809	75	60.1
Oswego	3922	95	100.0
Schmidt	2396	71	72.8
Windsor	1855	79	43.7

In addition, examination of fresh material during the seasons of 1931 and 1932 concurs with the previous findings. Measurements and critical examination were made under the dissecting microscope at regular intervals during the season. The results are given in table II. They, too, are in general agreement. It will be noted that there

is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later. Moreover, there is the tendency for the aborted embryos of the earliest-ripening varieties to be smaller than those of varieties which ripen a little later.

TABLE II

RELATION BETWEEN SEED AND FRUIT DEVELOPMENT AND ABORTION OF EMBRYOS IN SWEET CHERRY IN 1931

VARIETY	NO. OF DAYS BETWEEN FULL BLOOM AND FRUIT RIPENING	LENGTH IN MM. 37 DAYS AFTER BLOOM				NO. OF DAYS FROM FULL BLOOM TO FIRST INDICATION OF EMBRYO ABORTION	PROPORTION OF VIABLE SEED
		OVARY	NUCELLUS AND INTEGUMENTS	CELLULAR ENDOSPERM	EMBRYO		
Burbank	48	17	6.8 (collapsed)	4.0	3.1	31	None
Governor Wood	48	16	6.0 (collapsed)	5.2	4.0	34	None
Lyons	49	18	7.2	5.2	4.8	38	None
Knight	52	16	6.7 (collapsed)	6.2	5.9	35	None
Elton	56	19	7.9	6.0	5.3	37	None
Black Tartarian	55	15	7.3	5.8	5.2	43	Some
Black Eagle	60	14	7.2	5.8	5.2	50	Most
Schmidt	64	15	8.0	6.2	5.3	No abortion	Most
Black Republican	68	15	7.9	6.2	5.9	" "	Most
Mazzard	68	9	7.0	5.5	5.3	" "	All
Abundance	75	11	6.8	5.0	4.8	" "	All
Oswego	77	11	6.9	.....	4.8	" "	All

Discussion

The sudden acceleration in growth of the ovary, the nucellus and integuments, and the megagametophyte or the embryo sac which occurs at the time of fertilization is not necessarily a response to fertilization in itself. Such acceleration may begin 24 hours before pollen tubes can be found within the ovary, and therefore, before fertilization has been accomplished, or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished. On the other hand, this rapid growth may begin similarly with unfertilized ovules and continue for 7 to 10 days. It may also begin temporarily with ovules containing a normal endosperm development but no embryo, and with ovules containing an embryo but no endosperm.

Further evidence on this point is presented by emasculated and unpollinated flowers. They show a similar sudden and rapid growth of ovary, of nucellus and integuments, and of megagametophyte. The megagametophyte elongates, the synergids and megagamete remain at the micropylar end of the megagametophyte, and the fused polar nuclei maintain a position nearly midway the length. Such a condition is shown in figure 4, 7 days after full bloom. No fertilization has occurred, and the style has abscissed. However, no blossoms of this type develop to the ripe fruit.

These facts suggest that the initiation of the period of rapid growth is *coincident with* the time of fertilization, but not necessarily *initiated by* it.

CONNORS (**4**), in discussing embryo abortion in early-ripening varieties of peaches, says: "The probable cause of abortion is the fact that the enlargement of the drupe takes place at the same time as the final period of development of the embryo, due to a heritable character determining the period of ripening. The flesh becomes mature and ripe and abscission takes place before the development of the embryo is completed."

That the last part of this suggestion does not apply to the cherry varieties under discussion in this paper is shown by the fact that embryo abortion may occur 12 days prior to ripening of the fruit, and that it occurs at different dates in different varieties. KRAUS (**8**) has called attention to the fact that this arrest in the development of an embryo is also found in the case of certain matings of fowls and in some mammals.

Since there appear to be no histological nor developmental changes prior to embryo abortion in the early-ripening type of sweet cherry, except for the initiation of the second period of rapid pericarp development at the time the embryo and endosperm are making their most rapid growth, attention is naturally focused upon this relation; yet it is not possible to say which is cause and which is effect. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication.

As a general proposition, however, from the behavior of plants in

general, one would hazard the opinion that if there is any nutritional balance between embryo development and pericarp development, the abortion of the embryo is the factor which determines the rate and time of the second period of rapid pericarp development rather than the reverse. The suggestion is made that abortion of the embryo may not by itself be responsible for the dropping of a fruit. Possibly quite to the contrary, embryo abortion may be a varietal character which is responsible for the earliness with which some varieties ripen their fruit.

Not only is embryo abortion a characteristic of later waves of dropped fruits, therefore, as pointed out by KRAUS, BRADBURY, DETJEN, and others, but it is also a characteristic of some varieties of fruits which normally ripen their fruit on the tree.

From the standpoint of the plant breeder, the establishment of the fact that early-ripening varieties fail to produce seed with fully developed embryos means that in efforts to breed early-ripening sorts, an early-ripening kind should not be used as the female parent. There is the suggestion from the studies of embryo culture by LAIBACH (**9**), TUKEY (**19**), and others that some progress might be made in this direction. By culturing an embryo from an early-ripening type upon which another early-ripening variety had been used as the male parent, the chances would be increased of securing a new individual with a combination of genes for early-ripening from both male and female parent. Once a horticultural variety is produced, its perpetuation is assured by the usual vegetative methods of propagation, such as budding and grafting.

That various parts of the fruit and seed may be developing at one time and other parts at another is an interesting point. Instead of the ovary, the nucellus and integuments, the megagametophyte, the endosperm, and the embryo all developing at similar times with parallel growth rates, they develop at different periods with varying growth rates. In spite of this seeming complexity and variability, the factors governing development are remarkably constant. That is to say, if the size of any one part is known, it is possible to tell the relative development of any related part at that time. In other words, when the embryo is a given size, the endosperm development bears a constant relation to it, the nucellus, integuments, and the

pericarp as well. This applies not only to any one season but to different seasons in comparison with one another. The graphs of development may vary in steepness and length, depending upon the season, but the relation between developing parts is constant regardless of the season.

The period of retarded pericarp development following a second period of rapid development is a characteristic of drupaceous fruits. LILLELAND (10) suggests that the retarded growth may be ascribed to the competition for food substances used in forming the stone. He considers that the fact that this cyclic type of growth has not been found in pome fruits further substantiates this premise.

Factors underlying embryo abortion as determined from cross-breeding records

Some light is shed on the factors underlying embryo abortion by the plant breeding records of the New York State Agricultural Experiment Station. Over a period of 7 years they show a minimum of crosses involving early-ripening varieties of cherries as female parents. This is because practical plant breeders have been rewarded with no progeny from such crosses, and therefore have purely empirically stricken such crosses from their cross-breeding programs. The few crosses that have been made which involve early-ripening varieties, however, show that when such varieties of cherries are used as female parents, no viable seed is produced, even though the male parent in the cross is a late-ripening kind. On the other hand. when late-ripening varieties of sweet cherry are used as female parents, viable seed is produced. The use of an early-ripening variety as male parent does not alter the results.

Table III gives the results from 13 recorded crosses over a period of 7 years involving early-ripening varieties as either male or female parent. The number of days elapsing between full bloom and ripening of fruit is given in parentheses after each variety. Unfortunately, there are only two reciprocal crosses, namely, between Seneca (35) and Black Tartarian (55), and between Burbank (48) and Lyons (49), and both of these crosses involve early or early mid-season varieties. Yet the figures tend to show that the limiting factor in the production of viable seed is the nature of the female parent and not

the male parent, and that the problem concerns physiology and nutrition rather than genetics.

Correlation between ripening of fruit and abortion of ovule and embryo

It has already been indicated that there is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later, and that there is a tendency for the aborted embryos of the earliest-ripening varieties to be smaller

TABLE III

PERCENTAGE VIABLE SEED SECURED FROM CROSS-BREEDING IN WHICH BOTH EARLY AND LATE-RIPENING VARIETIES OF CHERRY ARE USED AS BOTH MALE AND FEMALE PARENTS

FEMALE PARENT	DAYS TO RIPEN FRUIT	MALE PARENT	DAYS TO RIPEN FRUIT	NO. OF FRUITS	PERCENTAGE VIABLE SEED
Seneca	(35)	× Burbank	(48)	2	0
Seneca	(35)	× Lyons	(49)	7	0
Seneca	(35)	× Black Tartarian	(55)	13	0
Seneca	(35)	× Abundance	(75)	13	0
Early Purple Guigne	(37)	× Burbank	(48)	10	0
Early Purple Guigne	(37)	× Lyons	(49)	21	0
Burbank	(48)	× Lyons	(49)	29	0
Burbank	(48)	× Black Tartarian	(55)	12	0
Lyons	(49)	× Burbank	(48)	28	0
Black Tartarian	(55)	× Seneca	(35)	456	8
Eagle	(60)	× Seneca	(35)	33	32
Eagle	(60)	× Republican	(68)	38	26
Giant	(65)	× Seneca	(35)	7	57

than those of varieties which ripen a little later. The question arises, therefore, as to any possible relation between fruits of varying degrees of ripeness on any one day on any given tree. That is, is there a correlation between accelerated pericarp development and abortion of embryo within the same variety?

In an attempt to answer this question, samples of fruits were collected each day during the season of 1932. The method was to take all the fruits on a given branch for each sampling. From 10 to 60 fruits were taken in each sample, involving a total of several thousand individuals, from which the data in tables IV and V were secured. The fruits from each variety were divided each day into various classes, according to degree of ripening as indicated by color.

It was at once apparent that there was a greater degree of variability in ripening among the early-ripening varieties than among the late-ripening kinds. The fruits of Early Purple Guigne, for example, within a span of 12 to 18 inches on a given twig, could be divided into green, greenish-yellow, yellow, red tinge, and half red.

TABLE IV

RELATION BETWEEN FRUIT RIPENING IN LATE-RIPENING VARIETY OF SWEET CHERRY AND DEVELOPMENT OF NUCELLUS AND INTEGUMENTS AND EMBRYO

PROGRESS IN RIPENING	LENGTH IN MILLIMETERS									
	JUNE 19	JUNE 20	JUNE 21	JUNE 22	JUNE 23	JUNE 24	JUNE 25	JUNE 26	JUNE 27	JUNE 28
	Total pericarp									
Green	12.86	.....	13.02	12.92	12.90	13.34	.....	.....	.....	.....
Red tinge	13.10	.....	13.10	13.16	.....	13.36	13.52	13.82	14.34	.....
Half red	.....	.....	.....	.....	13.82	.....	14.45	14.52	.....	.....
Full red	.....	.....	.....	.....	.....	.....	.....	.....	14.64	14.76
	Nucellus and integuments									
Green	7.76	.....	7.84	7.72	7.84	7.76	.....	.....	.....	.....
Red tinge	7.84	.....	7.80	7.76	.....	7.76	7.96	8.00	7.56	.....
Half red	.....	.....	.....	.....	8.12	.....	8.21	8.00	.....	.....
Full red	.....	.....	.....	.....	.....	.....	.....	.....	7.82	7.76
	Embryo									
Green	6.52	.....	6.88	6.94	6.80	7.04	.....	.....	.....	.....
Red tinge	6.80	.....	6.70	6.90	.....	6.96	6.60	7.12	6.84	.....
Half red	.....	.....	.....	.....	7.00	.....	7.30	7.28	.....	.....
Full red	.....	.....	.....	.....	.....	.....	.....	.....	7.04	7.12

On the other hand the fruits of Downer, on a given twig, were more uniform in development; that is, nearly all either in the green stage, the greenish-yellow stage, or the yellow stage, and so on. It was only with difficulty that various degrees of ripening could be secured from the late-ripening varieties of cherry, like Downer.

Likewise it was at once apparent from dissections that there was greater variation in size of embryo, endosperm, and nucellus and

integuments within the early-ripening varieties than in the late-ripening ones. The latter were strikingly similar in size and development on any given date.

TABLE V

RELATION BETWEEN FRUIT RIPENING IN EARLY-RIPENING VARIETY OF SWEET CHERRY AND DEVELOPMENT OF NUCELLUS AND INTEGUMENTS AND EMBRYO

Progress in ripening	Length in millimeters										
	June 5	June 6	June 7	June 8	June 9	June 10	June 11	June 12	June 13	June 14	June 15
	Pericarp										
Green	13.58	13.48	.....	.....	.....	.....	.....	.....	.....	.....	.....
Red tinge	13.77	13.81	14.40	14.73	.....	14.40	.....	.....	.....	.....	.....
Half red	.....	14.70	15.13	15.23	.....	14.64	15.32	.....	.....	.....	.....
Three-fourths red	14.22	15.26	15.80	.....	15.10	14.86	15.56	15.88	.....	16.22	.....
Full red	14.30	.....	.....	.....	15.56	.....	15.60	16.03	.....	16.41	17.16
	Nucellus and integuments										
Green	7.15	7.38	.....	.....	.....	.....	.....	.....	.....	.....	.....
Red tinge	6.91	7.13	6.80	6.56	.....	7.00	.....	.....	.....	.....	.....
Half red	.....	6.42	6.53	6.46	.....	6.40	6.96	.....	.....	.....	.....
Three-fourths red	6.36	6.40	6.30	.....	6.73	6.43	6.56	6.88	.....	6.84	.....
Full red	5.60	.....	.....	.....	6.60	.....	6.10	6.66	.....	6.46	6.96
	Embryo										
Green	3.41	3.66	.....	.....	.....	.....	.....	.....	.....	.....	.....
Red tinge	3.47	3.74	3.52	3.16	.....	4.65	.....	.....	.....	.....	.....
Half red	.....	3.06	2.90	3.90	4.96	4.68	5.54	.....	.....	.....	.....
Three-fourths red	2.90	3.63	3.70	3.48	4.73	5.13	5.08	5.44	.....	5.68	.....
Full red	1.60	.....	.....	.....	4.58	.....	3.83	5.63	.....	5.76	6.04

As might be expected, the larger fruits on any given date were farthest along in ripening, as shown in tables IV and V and also in figure 41. Yet all flowers bloomed within a span of 2 days, so that it is not possible to lay this difference to lateness in blooming of some flowers as compared with others on the same branch or twig.

Variation in time of pollination or in time of fertilization could be responsible for the variation; but if this is so, it is difficult to understand why the fruits of a variety like Downer developed uniformly, while those of a variety like Early Purple Guigne developed irregularly.

An examination of the nucellus and integuments of the late-ripening variety, Downer, shows this same relation. Larger nucellus and

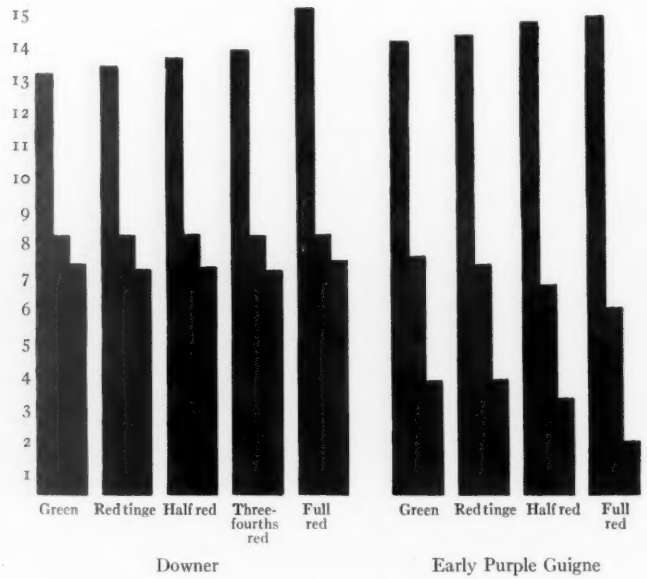


FIG. 41.—Relation between size of pericarp and embryo and nucellus and integuments in fruits of different degrees of maturity picked on the same day. In each column, left-hand section represents the pericarp, center section the nucellus and integuments, and right-hand section the embryo.

integuments are found with larger and riper fruits (table IV). On the other hand, the early-ripening variety, Early Purple Guigne, shows an exactly inverse relation. The largest and most nearly ripe fruits on any given date have the smallest ovules (table V). This fact is most readily seen in figure 41.

Likewise with the embryos, the largest embryos are found in the

largest and ripest fruits of the late-ripening variety, Downer, on any given date, thus correlating large fruit-size and advanced ripening with large size of nucellus and integuments and large size of embryo. And here again, in the case of the early-ripening variety, Early Purple Guigne, the relation is inverse. The smallest embryos are found in the largest and ripest fruits collected on any one day. Yet this inverse relation is not so regular as in the case of the nucellus and integuments. The embryos are often larger, proportionately, than the nucellus and integuments. This suggests that an embryo may continue development for a time independently of development of the nucellus and integuments, as BRADBURY has indicated in abscissed fruits of sour cherry. Figure 8 also shows an embryo which has developed while still contained within the integuments of an abortive seed, in this case producing six true leaves.

The inference to be drawn from these facts is that a check in the development of the nucellus and integuments precedes a check in the development of the embryo. Furthermore, since the embryo seems often to continue development after arrest of other parts of the seed, the underlying cause of embryo abortion is pushed back one step further, namely, to whatever is responsible for cessation of development of the nucellus and integuments. That this is nutritional seems likely, but not necessarily proved by these data.

There is, however, no light shed on whether the check in development of the ovule is due to rapid development of the pericarp, or whether rapid development of the pericarp is in turn due to an abortion of the ovule and the developing embryo. All that can be said is that in varieties of cherries which normally abort the embryo, the largest and ripest fruits on any one given day normally contain the smallest nucellus and integuments and also the smallest embryos.

Artificial culture of normally abortive cherry embryos

Further evidence that the problem is one of nutrition is given by the artificial culture of cherry embryos (**19**) which was suggested by the study here reported and by the work of LAIBACH and others on the culture of plant embryos. It was reasoned that since there appeared nothing structurally abnormal in aborting embryos, and since embryos in aborted seeds and fruits often continue to de-

velop for a time after abscission, that if given the proper conditions for growth, these aborting embryos might continue to develop. That this has been the case, and that seedlings with true foliar leaves have been grown by this method from normally aborting embryos, show that lack of proper nutritive conditions rather than any hereditary lethal factor has been responsible for failure of early-ripening varieties to produce viable seed.

Summary

1. Both nurserymen and plant breeders experience difficulty in securing seedlings from seed of early-ripening varieties of sweet cherry (*Prunus avium*).

2. Critical developmental studies are reported for a late-ripening type, the variety Downer, and an early-ripening type, the variety Early Purple Guigne.

3. Comparison is made between the development of these two varieties during the seasons of 1930, 1931, and 1932, and also with other varieties: Burbank, Governor Wood, Lyons, Knight, Elton, Black Tartarian, Black Eagle, Schmidt, Napoleon, Black Republican, Abundance, Oswego, and Mazzard.

4. The embryogeny of the sweet cherry is essentially the same as described by BRADBURY for the sour cherry, and as described by PÉCHOUTRE, RUEHLE, and others.

5. The megaspore mother cell is distinguished 10 to 14 days before full bloom, at about the time the flower blossoms have emerged from the bud and have just separated in the cluster. Reduction division occurs several days later. The megagametophyte is formed 3 to 7 days before full bloom, about when the petals are showing.

6. The ovule lengthens but slightly between the 55th day before full bloom and the time of full bloom. The non-functional ovule is arrested in development just prior to full bloom, but the megagametophyte in the aborted ovule may persist unelongated, but otherwise complete, for 10 to 14 days longer.

7. Ovary development parallels that of the nucellus, in which increase in length is slight from the 55th day before full bloom until full bloom.

8. At the time of fertilization there is a sudden acceleration in

growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac, which continues for a period of 17 days after full bloom. The nucellus and integuments reach nearly maximum size, and the embryo sac reaches nearly to the chalaza.

9. Development of the embryo and endosperm is much delayed. The endosperm remains free-nuclear until the 9th day after full bloom, and is then cellular only for a short distance. Subsequent development is by cell division progressively from the micropylar end toward the chalazal end, and from the periphery toward the center. The free-nuclear endosperm in the chalazal end of the embryo sac does not become cellular.

10. Seventeen days after bloom rapid pericarp development ceases almost as abruptly as it begins and a period of retarded growth follows. The period of retarded pericarp development continues 8 days for the early-ripening variety and 14 days for the late-ripening kind. During this period the embryo and cellular endosperm begin a sudden and rapid development.

11. A second period of rapid pericarp development follows the period of retarded development. It begins 31 days after full bloom for the late-ripening type and 25 days after bloom for the early-ripening type. The embryo of the late-ripening type has developed to a length of 3 mm., whereas the embryo of the early-ripening type is only 0.52 mm. in length.

12. The embryo of the late-ripening variety during this period develops to maximum size, surrounded by a thin layer of endosperm and a line of nucellar tissue, all contained within the two integuments, to give the viable seed characteristic of this type of fruit. On the other hand, in the case of the early-ripening variety, four waves of embryo abortion occur at this time. The nucellar tissue collapses, the integuments shrivel, and the characteristic abortive seeds of early-ripening varieties are the result.

13. Embryo abortion is observed as a sudden arrest in development of the embryo. No apparent structural variation has been observed in abortive embryos, with the exception of the tendency for such embryos to assume a flattened shape at the tips of the cotyledons, with the cotyledons overlapping each other.

14. The first indication of irregularity in development occurs at the region of contact between the nucellar tissue and the developing cellular endosperm. At this point there is a loss of turgor in the nucellar tissue just prior to the general collapse of the nucellus and integuments.

15. Although abortive embryos are frequently associated with dropped fruits, in this case they are associated with normally ripening fruits. Abortive embryos need not necessarily be associated with poor pollination, sterility, incompatibilities, or even nutrition.

16. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication. Although all parts do not develop at the same rates and during the same periods, yet they bear the same relation to one another even in different years.

17. The sudden acceleration of parts at the time of fertilization is coincident with the time of fertilization but not necessarily initiated by it. Emasculated and unpollinated flowers show the same sudden rapid development of ovary and of nucellus and integuments, and the marked elongation of the megagametophyte as in the case of hand-pollinated and fertilized blossoms, but do not continue to the formation of ripe fruit.

18. The effect of emasculation and bagging is to retard the development of all flower parts by from 4 to 6 days.

19. In the central zone of the nucellus, reaching from the chalaza to the base of the megaspore mother cell and the megagametophyte, a core of elongated cells is formed which suggests a conductive system, and which is the tissue through which the megagametophyte and embryo sac elongate in their subsequent development.

20. Early-ripening varieties of sweet cherry should not be used as female parents in the breeding of new varieties. Breeding records involving late-ripening and early-ripening varieties of cherry show no progeny when early-ripening varieties are used as female parents, but do show progeny when used as male parents, indicating that the problem of production of non-viable seed is not genetic.

21. Among varieties of cherries which normally contain abortive embryos, the earliest-ripening ones contain the smallest embryos.

22. The largest and ripest fruits on a given branch of a late-ripening variety on any one day contain the largest ovules and embryos. This relation is exactly the reverse for early-ripening varieties, in which the ovules and embryos are smallest in the earliest-ripening fruits. These facts indicate that a check in the development of the nucellus and integuments precedes a check in the development of the embryo.

23. Further evidence that the problem is one of nutrition is given by successful artificial culture of normally abortive embryos.

The writer wishes to express his indebtedness to the Department of Botany of the University of Chicago, under whose direction the work was conducted. He wishes also to express his appreciation to Dr. B. R. Nebel, Karl Brase, and Mrs. Marion Barrett, and especially to Dr. Mable Ruttle Nebel (Mrs. Nebel), for their assistance during various stages of the work.

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ORIGIN AND DEVELOPMENT OF TISSUES IN EQUISETUM SCIRPOIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 440

MARION A. JOHNSON

(WITH PLATE VIII AND TWENTY-NINE FIGURES)

Introduction

Equisetum scirpoides is the smallest representative of the genus *Equisetum*. It is a circumpolar species extending in North America from Labrador to Pennsylvania, westward to Illinois and thence northwestward into Alaska. The general appearance of the plant is that of a mat or dense tuft, formed from a tangle of rhizomes which give rise to aerial shoots bearing branches from their lower nodes. The stems are seldom straight, being twisted and intertwined, thus contributing further to the matlike habit.

The internal stem structure is similar to that characteristic of the genus, in that there is a formation of supranodal wood from which the bundles below alternate with those above, a loss of protoxylem with the resulting carinal canals, and a cortex with chlorophyll-bearing tissue and prominent vallecular cavities. The bundles and vallecular cavities are typically three in number, although in the rhizome four not infrequently occur. The pith is not disrupted as in the larger species but remains intact.

Equisetum has long attracted the attention of botanists on account of its structure and phylogenetic relationship with Calamites and Sphenophyllales. Despite this interest, no complete account of the origin and development of tissues for *E. scirpoides* has apparently yet appeared. It is the purpose of this paper to present such an account.

Since a relatively small portion of the work on *Equisetum* is concerned with *E. scirpoides*, only brief mention of the literature will be made. The older accounts up to 1864 are ably summarized by DUVAL-JOUVE (**17**), while the more recent papers have been referred to by BROWNE (**7**). Since then BROWNE (**8–11**) has made further

anatomical studies on the cone and stem. In 1920 BARRATT (**4**) presented additional material on the vascular system. The gametophytes of several species have been studied by WALKER (**36, 37**) and by ASAHINA (**1**). SCHAFFNER (**24–26**) dealt with the problem of evolution in *Equisetum* and with the geographic distribution of the species in relation to their phylogeny.

Materials and methods

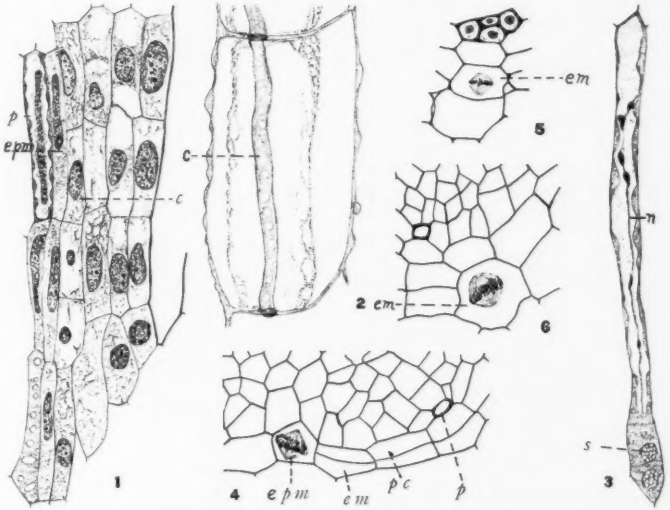
Collections of whole plants were made by Miss MILDRED FAUST of Syracuse University from an Arbor Vitae swamp near Syracuse, N.Y. Additional material was kindly supplied by the director of the Brooklyn Botanic Garden. Vegetative tips and roots were obtained in abundance from plants growing in soil and from others placed in water. A formalin-acetic-alcohol mixture was found to be a satisfactory killing agent; plasmolysis was slight and mitotic figures, especially of the metaphase, were numerous. Siliceous materials were removed by treatment with hydrofluoric acid. Both the usual histologic technique of dehydration and imbedding in paraffin and the butyl alcohol method as proposed by ZIRKLE (**39**) were used. The latter gave somewhat better results, but in either case cutting was facilitated by soaking in water for several days. Safranin and light green, and safranin and fast green, were found to make excellent staining combinations. Either light green or fast green proved to be valuable in differentiating the phloem before the other vascular elements could be recognized with certainty.

Observations

APICAL CELL AND ITS DERIVATIVES

The axis of the aerial shoot is terminated by the free surface of a pyramidal apical cell which by anticlinal walls cuts off segments from its three lateral faces. Transverse sections taken immediately below the apical cell show three nearly equal segments extending to the center. As in the ferns, each of these is divided unequally by an oblique wall, forming in all six sextants so placed that the larger meet at the center and alternate with the three smaller ones. A few exceptions to this regular arrangement were found in which the major sextants did not alternate but were cut from adjacent sides of the

original segments. Tangential walls soon divide each of the large sextants, forming three inner cells which give rise to the pith. From this point on, both radial and tangential divisions are made in such rapid succession that it is difficult to trace the order of their appearance. Median longitudinal sections through the stem tip show, as reported by REESS (**23**), that each segment from the apical cell



FIGS. 1–6.—Fig. 1, portion of longitudinal section of stem showing limit of stele and cortex: *p*, phloem; *epm*, endodermis-pericycle mother cell; *c*, cortical cells; ×500. Fig. 2, longitudinal section through endodermis at node: *c*, Casparian strip with vacuoles; ×2000. Fig. 3, sieve tube showing: *s*, lateral sieve plates; *n*, disintegrating nucleus; ×1280. Fig. 4, transverse section of stem showing endodermis-pericycle mother cell giving rise to pericycle and endodermis mother cell: *pc*, pericycle; *em*, endodermis mother cell; ×500. Figs. 5, 6, portions of transverse sections of stem showing endodermis mother cell dividing to form endodermis and layer of cortex; ×500.

undergoes anticlinal and then periclinal division, forming two tiers of two cells each, the upper ultimately developing into the node and the lower into the internode. The inner cell of each tier gives rise to pith while the outer ultimately produces cortex and stele, although it is impossible to determine whether the separation of these tissues is accomplished by the first periclinal division. At first the

derivatives are alike in appearance, but at the third or fourth node from the tip two distinct regions can be seen (fig. 1). The cells in the outermost region are approximately one-half as long and twice as broad as those in the innermost one, possess spherical nuclei, and have large vacuoles and a fibril-like cytoplasm. This doubtless is the cortex. The innermost region on the other hand is made up of long narrow cells with elongated nuclei and dense granular cytoplasm free from large vacuoles. Subsequent differentiation proves this to be the stelar tissue.

Pith

The pith, which is cut off from one of the original segments of the apical cell by the first periclinal wall, divides at once by both periclinal and anticlinal walls. This early activity extends through the second or third nodes, below which general maturity is evident. The cells are not disrupted as in the larger species, but have their walls thickened with a green staining material and supplied with numerous simple pits.

Phloem

As a rule the protophloem is the first of the vascular elements to be recognized. It is differentiated from a prominent procambial strand which makes its appearance in the node, and then extends down into the internode and up into the leaf, where near the tip it is reduced to several cells closely resembling the remaining leaf parenchyma. The order of differentiation is the same as that for the procambial strand, and when complete forms a continuous connection throughout the leaf, node, and internode. At maturity the protophloem caps the vascular strand as one or two layers of small, thick-walled cells which have somewhat the appearance of fibers. Careful examination of the lateral walls reveals the presence of sieve plates with very small pores (fig. 3).

Stages in the development of the protophloem show a gradual elongation of the cell, accompanied by the deposition of thickenings on the walls and a decrease in the density of the cytoplasm. The nucleus keeps pace with the elongating walls until it is hardly more than a ribbon of nuclear granules surrounded by a membrane. The elongation of the nuclei is striking, lengths of 230μ being not uncom-

mon. In some cases the nucleus loses its membrane, takes on a slimy appearance, and displays a few spindle-shaped bodies which stain red with safranin (fig. 3). In still other cells the nucleus disappears entirely, which seems to be the normal condition at maturity.

Metaphloem if present is not distinguishable from the phloem parenchyma, which in turn cannot be differentiated from the xylem parenchyma.

Xylem

The protoxylem first becomes lignified in the upper part of the internode, but soon develops throughout the internode and ultimately into the node, making connection with the leaf trace below. A second center of lignification occurs high in the leaf sheath, extending both up into the leaf and down into the sheath until union is made with the protoxylem of the stem. The order of differentiation in the leaf trace seems to differ somewhat from that described by Barratt (**4**), who found that lignification in *E. arvense* began at the tip. The individual protoxylem elements consist of tracheids with spiral or annular thickenings, except in the node, where transitions from spiral to spiral-reticulate and even reticulate markings occur, as has been noted by Vidal (**35**) in *E. palustre*. Elongation in the internode is so rapid that the protoxylem is early disrupted with the formation of the characteristic carinal canals.

In vigorously growing shoots the metaxylem is differentiated in the sixth internode from the tip. It consists of from one to seven cells flanking the protoxylem, and is continuous throughout the node, internode, and leaf trace. The greatest display, however, is found at and above the node, where it forms a band of supranodal wood which may be two to four cells in thickness (fig. 31). The tracheid walls are conspicuously marked with scalariform thickenings and multiseriate pits.

The order of lignification is endarch in the stem and mesarch in the leaf, as reported by Eames (**18**).

Endodermis and pericycle

The endodermis is composed of a single layer of cells completely inclosing the stele and continuing through both the node and internode. It also surrounds the leaf traces, extending up into the leaves

until it comes in contact with the epidermis, which in some instances is so complete that a Casparian strip is common to both (fig. 12 *d*). The pericycle is generally one layer in thickness, although occasionally it may be doubled. This, however, was found to be a very rare occurrence. No particular activity in forming new tissues was found, except that not infrequently a pericycle cell matures as metaxylem adjacent to the endodermis.

The young pericycle and endodermis cells are of the same length; later those of the latter begin to divide by cross walls, so that at maturity its cells are often three or more times as long as those of the pericycle. Stages in the development of the Casparian strip were not observed. It was found, however, to be composed of a delicate band of homogeneous material with one or more rows of tiny vacuoles (fig. 2).

The problem of determining the origin of the endodermis is complicated by the alternation of the vascular bundles at the node. By using the protophloem as a point of reference, and knowing that at maturity the pericycle and endodermis are each one layer in thickness, it is possible to locate the endodermis and pericycle long before the Casparian strips are developed. Cross-sections show the young pericycle cells paired with the stelar elements, and in turn the endodermis paired with the cortex as well as with the pericycle. This situation suggests that the endodermis might be derived from either stele or cortex, or perhaps from both. Definite mitotic figures were discovered which show a single cell (fig. 4 *epm*) giving rise to both pericycle and endodermis mother cell. The endodermis mother cell (figs. 5, 6 *em*) in turn forms the endodermis proper, together with a layer of cortex which may also divide. The question arises as to whether the pericycle-endodermis mother cell belongs to stele or cortex. An examination of longitudinal sections through a bundle two or three nodes from the apex reveals a single cell (fig. 1 *epm*) just outside the protophloem which, on account of its length, elongated nucleus, and finely vacuolated cytoplasm, is regarded as belonging to the stele. This cell corresponds with *epm* of figure 4, which upon division gives rise to pericycle and endodermis mother cell; thus it follows that the pericycle, endodermis, and at least one layer which is regarded as cortex is stelar in origin.

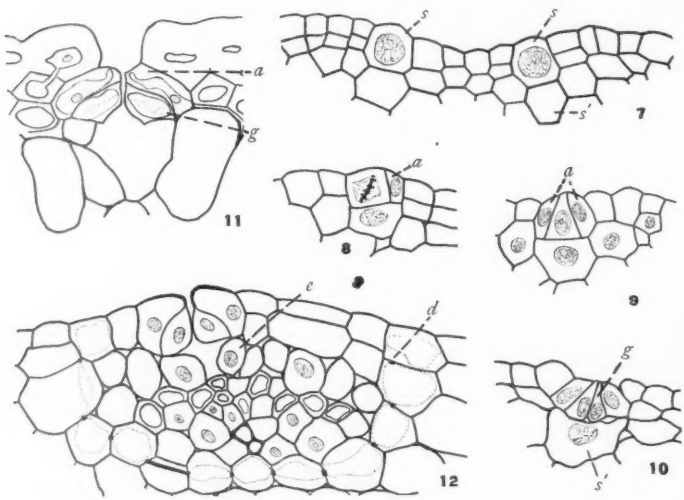
Epidermis and cortex

The epidermis is made up of long, somewhat irregular, close-fitting cells with exceedingly thick walls. Siliceous materials are deposited in and on the walls, especially the outer tangential wall which has an undulating appearance due to uneven thickening. A single uniform hypodermal layer occurs in the furrows of the stem, but is doubled on the ridges, where the cells are larger and the walls thicker.

In the furrows of the stem and leaf sheath the epidermis is interrupted by two prominent rows of stomata, whose origin can readily be traced in transverse sections taken immediately above the nodes of actively growing stems. The earliest recognizable stage consists of a cell which is much larger than its neighbors. A tangential division results in the formation of two daughter cells (fig. 7 *s*, *s'*). The outer cell (*s*), by two slightly oblique radial walls, as shown in figures 8 and 9, gives rise to the accessory cells (*a*) flanking the true stoma mother cell. This mother cell is divided equally by a radial wall to form the guard cells (fig. 10 *g*). The substomal cell (*s'*) keeps pace with the enlarging stomal unit until organization is complete; then a cleavage is noted in the wall common to it, the accessory cells and the guard cells (fig. 10). Separation of the walls continues until the outer face of the substomal cell has been freed. The wall between the guard cells splits also, forming the stoma. During subsequent growth the substomal chamber is enlarged and the epidermis overarches the stomal unit so that it lies in a shallow pit (fig. 11). This encroachment by the epidermal cells displaces the accessory as well as the guard cells, and they become oriented with the long axis tangent to the circumference of the stem. At maturity the accessory cells are covered with siliceous tubercules and the guard cells have the well-known bands found in the genus.

On the upper surface of the leaf, directly over the midrib, there are two or three rows of stomata which differ from those found elsewhere (fig. 12). The accessory and guard cells are only slightly cutinized, and the cells adjacent to the substomal cavity are free from chloroplasts and have the appearance of epithem tissue, as seen in hydathodes. The hydathodal nature is further suggested by the close association with the vascular bundle, the substomal chamber open-

ing upon the xylem, and the presence of an endodermis which makes contact with the epidermis on either side of the stomata-bearing region, thus surrounding the whole unit and separating it from the neighboring chlorophyllose cells. That these stomata are hydathodes can easily be demonstrated by placing plants in a saturated atmosphere. Under such a condition drops of water appear on the upper surface of the leaves. Since there are no other natural open-



FIGS. 7–12.—Figs. 7–11, transverse sections through stems showing stomata in various stages of development: *s*, mother cell of stomal unit; *s'*, substomal cell; *a*, accessory cells; *g*, guard cell; ×375. Fig. 12, transverse section of leaf showing water stoma: *e*, epithem tissue; *d*, Casparian strip common to wall of endodermis and epidermis; ×375.

ings above the leaf sheath, it is evident that the stomata in question have a secretory rôle. If the endodermis serves here as a waterproof layer, this is a striking example of an arrangement for the exudation of water without flooding the intercellular spaces of the surrounding mesophyll.

The cortex proper is composed of four or five layers of radially arranged chlorophyll-bearing cells. Stages in maturity first occur in the upper part of the internode, and extend downward until a more

or less permanent meristematic region is reached at the base. This basal meristem contributes to both stele and cortex. Three prominent vallecular cavities, whose formation is accomplished by separation and rupture of the parenchyma cells under each furrow, traverse the internode.

Leaf and leaf trace

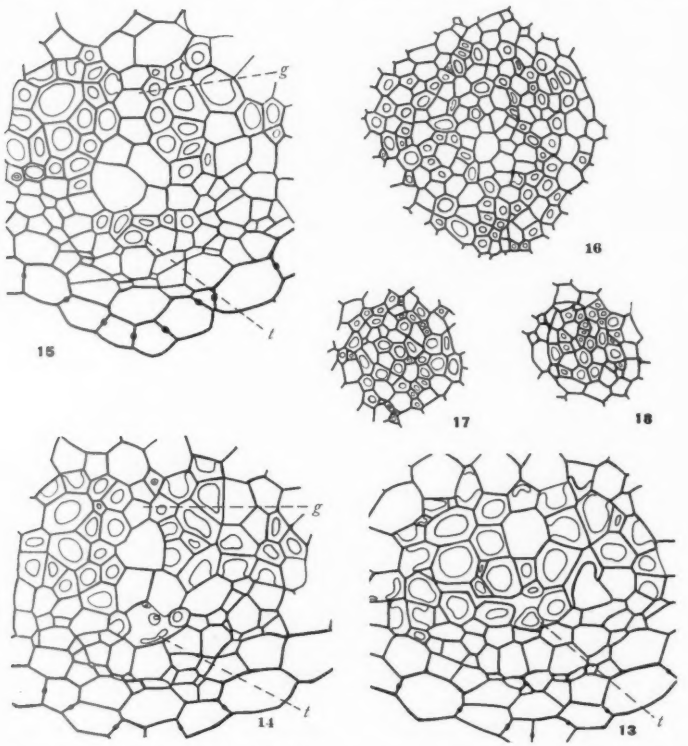
The origin of the leaves can be traced to apical cells which are equidistant from each other on a crown of meristematic tissue a few segments below the apical cell of the shoot. This crown of tissue arises from division and growth in the region of the node, and develops into the sheath bearing the three reduced leaves. Each leaf contains a single vascular bundle, with the exception of the first whorl of the lateral branches which lacks a vascular supply. The chlorophyllose tissue is poorly developed even though the leaves are persistent.

The leaf traces take their origin from the node, where each of the bundles begins to diverge. A careful study of transverse sections in serial order reveals, in some cases, a gap in the supranodal wood immediately above the trace (figs. 13–15). In other instances the gap may be closed by a single metaxylem cell, or there may be no evidence whatever of a break in the xylem. Communication between pith and cortex is never made because of the presence of a well-developed endodermis. It has been definitely determined that these gaps are not due to lack of maturity; some of the most striking ones were seen in old stems which were fully developed and were even bearing branches from the upper nodes.

Branch

The branches, as previously noted for *Equisetum*, arise in the axil of the leaf sheath between the leaves instead of in the leaf axil as in other plants. The mother cell of the branch, which is in the shape of a rectangular prism, is oriented with its long axis at right angles to the leaf sheath and is the outermost cell from a series of periclinal divisions, being thus truly exogenous. Later its shape is modified by the growth of the leaf sheath, so that it is broader at the base than at the exposed tip. The first division occurs parallel to the longitudinal axis, forming two cells of about the same volume. The upper one

gives rise to a row of isodiametric cells, while the lower one becomes the branch initial proper. By further growth the initial assumes the shape of the original mother cell, with the lower part of the outer face



FIGS. 13–18.—Figs. 13–15, series of transverse sections through mature stem showing departure of leaf trace and reduced condition of metaxylem: *t*, leaf trace; *g*, gap in metaxylem; ×475. Fig. 13, bundle before departure of leaf trace. Fig. 14, leaf trace still attached to bundle; note gap in metaxylem. Fig. 15, leaf trace free from bundle; note persistent gap. Fig. 16, transverse section through cone axis immediately below first whorl of sporangiophores showing arrangement of xylem; ×350. Figs. 17, 18, same as fig. 16, except from above first and second whorl of sporangiophores respectively; ×350.

somewhat rounded, owing to the pressure exerted by the developing leaf sheath. The first division of the initial itself is perpendicular

to its longitudinal axis, cutting off a basal cell which divides repeatedly so that its progeny cannot be distinguished from the cells lying just beneath. Continued division in this region forces the initial upward, forming a much sharper angle (fig. 19) with the main stem than is found in the other species of *Equisetum*. A series of three divisions now cuts up the initial into as many segments, forming at the same time the pyramidal apical cell which divides in the same manner as does the apical cell of the main axis (fig. 20).

The base of the leaf sheath grows up around the branch, covering it completely, which makes it necessary for the branch to force its way through several layers of tissue before appearing outside (fig. 21). It was not determined whether mechanical force or digestive activity or a combination of both is employed in breaking through the sheath.

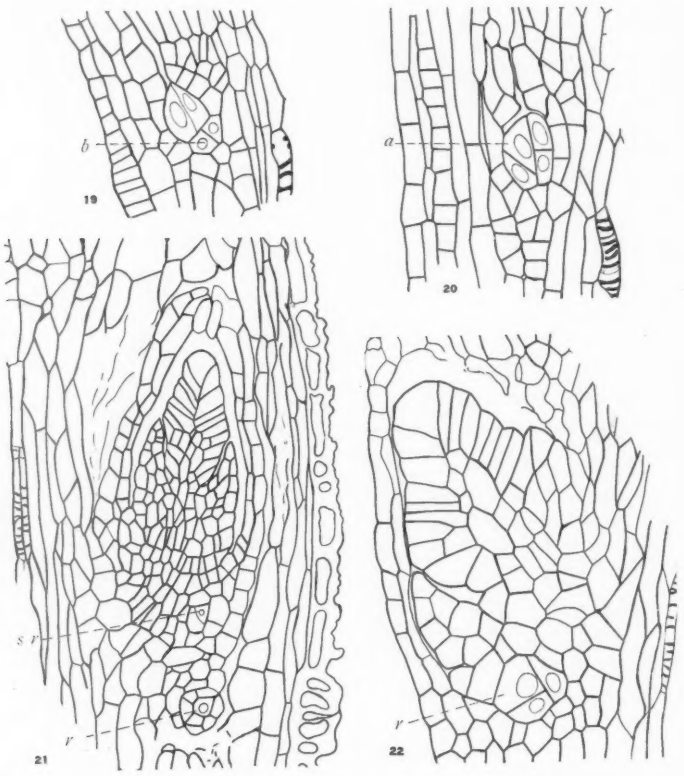
The occurrence of dormant branches is not at all uncommon; in fact they can be found at practically every node. In the lower ones they are well developed (fig. 30); in the upper ones, however, growth appears to be arrested but organization is complete. Their viability may be demonstrated by cutting stems into lengths of one node each and placing in water. In this way one or more branches may be induced from each node.

No evidence of a branch gap could be found. The departure of the trace leaves the supranodal wood undisturbed. In figure 31 the unbroken cylinder of xylem at the point of departure of the trace can clearly be discerned.

Rhizome

The rhizome, while similar in general structure to the aerial shoot, exhibits a somewhat greater diameter, usually four vascular bundles, longer internodes, and four well-developed leaves in each whorl instead of three. The origin and development of the various tissues seems to be the same as in the aerial stems. One noteworthy feature is the reduction of metaxylem in the internode so that each flank consists of from one to four cells. It is not uncommon to find bundles in which the metaxylem of one flank is entirely absent for several millimeters. In such cases the other flank remains normal (fig. 28). No instance was found in which a flank was absent throughout the

length of an internode. The reduction of the metaxylem in the internode has been noted previously. QUÉVA (**22**) states that it may sometimes be completely absent from the internodes of rhizomes of *E.*



FIGS. 19–22.—Fig. 19, longitudinal section through young branch; initial has cut off two segments (one in plane of the paper); note basal cells (*b*); ×340. Fig. 20, same as figure 19 except that initial has cut off three segments and formed a typical pyramidal apical cell (*a*); ×340. Fig. 21, longitudinal section of completely formed branch: *r*, first root; *sr*, later developed root; ×180. Fig. 22, *E. arvense:* longitudinal section of branch showing origin of root: *r*, root initial; ×340.

maximum and *E. limosum*. BROWNE (**10**) is of the opinion that the reduction in metaxylem is correlated with the relatively small number

of branches that are developed. While this may be true, it must be remembered that in *Equisetum* each node is supplied with roots, which doubtless reduces the movement of water through the internodes to a minimum. Furthermore, the roots are not attached to the rhizome proper but to buds, so that the aerial shoots have their own root supply almost from the beginning, and are therefore practically independent of the rhizome. It would seem, therefore, that reduction of the metaxylem is not only correlated with the small number of branches but also with the independence of the node and aerial shoots. In either event the transpiration stream between internodes would be small, which might in itself bring about reduction of the metaxylem. Certainly the reduction of xylem under such conditions would be no hardship in the plant. It is interesting to note that reduction is confined to the xylem, while the phloem remains unchanged.

The occurrence of tylose-like growths in the carinal canals is a unique feature of the rhizome and one which has been reported but twice previously for the genus (SYKES **32**, STRASBURGER **30**). The parenchyma cells bordering the canals proliferate to such an extent that they are completely filled; in cross-section they appear as patches of irregular cells with large nuclei and cytoplasm heavily laden with starch grains (fig. 32).

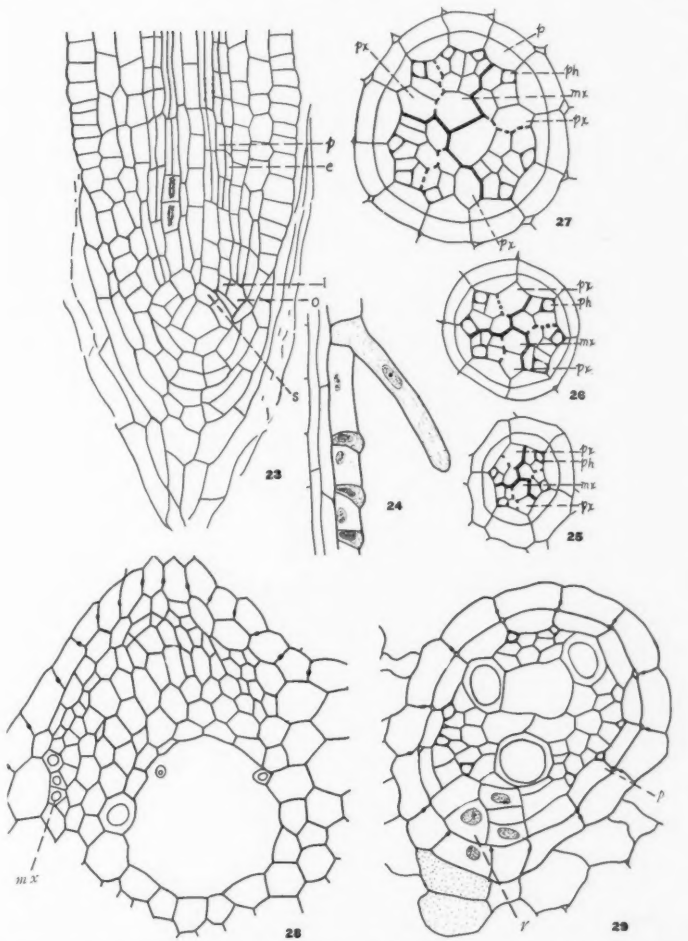
ROOT

The roots are about 0.5 mm. in diameter and often reach a length of 20 cm. or more. They are brown in color, and are covered for several centimeters back from the tip with a dense growth of root hairs which remains intact long after the tissues have reached full maturity. A prominent root cap protects the apical region.

The roots in the mature sporophyte arise only in connection with the lateral branches, this being true for both the aerial stem and the rhizome. The root initial may be traced to the lowest segment in the first series of three cut from the apical cell. This segment divides by anticlinal and periclinal walls, as described for the stem tip, so that two tiers of three cells each result. The upper tier develops into node and leaf sheath, while the two outer cells of the lower add to the base of the leaf sheath and the innermost cell enlarges and becomes the root initial. The initial is isodiametric in shape, has a large nu-

cleus and dense cytoplasm; and by three intersecting walls gives rise to a pyramidal apical cell whose three lateral faces contribute to the root proper, while the outer one forms the root cap. This method of root formation was found to be true of *E. arvense* also (fig. 22). The first root in each of these species is clearly endogenous; other roots may arise at the base of the leaf sheath as shown in figure 21. Their origin was not determined, however, although it also is definitely endogenous as to position.

The tissues of the root, as has been stated, are derived from an apical cell with four cutting faces. The outer segment is early divided by radial walls into four cells, from which the massive root cap is developed by subsequent divisions. Each of the three internal segments divides by a periclinal wall, separating stele and cortex, and a second periclinal wall soon follows, cutting off the epidermis (fig. 23). The epidermal cells divide at right angles to the long axis of the root, and ultimately become much elongated, with the exception of the root hair initials. These may be early recognized by their small size, large nuclei, dense cytoplasm, and their failure to elongate with the other epidermal cells. Back of the region of elongation they become papillate and grow out into characteristic root hairs (fig. 24). The remaining cortical cell after separation of the epidermis divides periclinally. The outer cell (fig. 23 *o*) develops into typical cortical tissue some four or more cells in thickness; two or three of the external layers ultimately have much thickened walls (fig. 35). Prominent intercellular spaces occur adjacent to the endodermis, so that the stele appears to be suspended by the inner layer of cortical cells (fig. 35). The inner cell (*i*) gives rise to pericycle and endodermis (figs. 23, 33). At maturity the endodermis is marked with the characteristic Casparian strips; while the pericycle, despite its cortical origin, is identical with that of the stem except that its cells remain paired with those of the endodermis. Lateral roots, as in other plants, have their origin in the pericycle, a single cell of which increases in size and divides three times, forming a pyramidal root initial with its base adjacent to the endodermis (fig. 29 *r*). Both the endodermis and cortex opposite the initial are stimulated to divide. Growth in these tissues, however, is not rapid enough to accommodate the young root, and it soon breaks through the cortex.



FIGS. 23–29.—Fig. 23, longitudinal section of root showing origin of tissues: *i*, inner cortical cell from original segment; *o*, outer cortical cell from same; *e*, endodermis; *p*, pericycle; *s*, first stelar cell from original segment; ×275. Fig. 24, longitudinal section through epidermis of root showing root hair and three root hair initials; ×180. Figs. 25–27, diagrammatic representation of central cylinder of root showing tissues contributed by each of the six sextants: *e*, endodermis; *p*, pericycle; *ph*, phloem; *mx*, metaxylem; *px*, protoxylem; ×375. Fig. 28, transverse section from internode of rhizome showing bundle with one metaxylem flank (*mx*); ×300. Fig. 29, transverse section through root showing origin of rootlet from pericycle: *p*, pericycle; *r*, rootlet initial; ×300.

The stele is developed from the inner cells (fig. 23 *s*), cut from the three original segments, and can best be followed in transverse section. Each cell is divided by a slightly oblique radial wall, forming two unequal daughter cells, the larger of which extends to the center. This is the familiar sextant stage described for other species of *Equisetum* and the pteridophytes in general. Almost at once each of the large sextants divides by a tangential wall, the three inner cells thus formed generally remaining undivided, even in the oldest roots (figs. 27, 34, 35). From this point on two distinct types of stelar development can be recognized. The first type, shown semidiagrammatically in figure 25, is found in the smaller roots. Here one of the small sextants fails to divide and matures as a protoxylem cell; each of the other two contributes a protophloem tube and two or three parenchyma cells. Each of the two large sextants forms a central cell, which remains as parenchyma, a protophloem tube, and two parenchyma cells. The remaining large sextant divides once, forming an inner lignified metaxylem cell and a protoxylem cell. The protophloem tubes are nearly always accompanied by two parenchyma cells, which might be considered as phloem parenchyma except that occasionally one of them adjacent to a protoxylem cell becomes differentiated into protoxylem. This type of root is reported by CHAUVEAUD (14) in *E. ramosissimum*. In somewhat larger roots an increase in the number of protophloem tubes frequently occurs (fig. 26). The small sextant contributing protoxylem and the large one giving rise to both protoxylem and metaxylem remain unchanged. The second small sextant contributes an additional phloem unit, and the third contributes two internal parenchyma cells. Likewise the third large sextant forms an additional protophloem tube and one parenchyma cell.

The second type of development was found in the largest roots (fig. 27), each of the small sextants differentiating to form a protoxylem cell, a phloem unit, and usually an internal parenchyma cell; while each of the large sextants contributes a metaxylem cell, two phloem units, and from one to several internal parenchyma cells. This striking equality in contribution from similar sextants seems not to have been noted in other species of the genus.

Cone

The reduced size of *E. scirpoides* is seen in the cone as well as in the other organs. The largest cones are rarely over 10 mm. in length or 2 mm. in diameter. The fertile sporophores consist of three or four whorls of three members each; a final sterile whorl is imperfectly fused into a hard protective cap. Basipetal protection is furnished the young cone by three large persistent leaves from the stem. The collar or annulus is present, but is much reduced and devoid of vascular tissue. When the spores have reached maturity, the internode between the protective leaves and the first whorl of sporangiophores elongates, thrusting the cone forward and slightly to one side, thus facilitating spore dispersal.

The vascular bundles at the base of the cone, as would be expected, are identical with those of the stem. As the first whorl of sporangiophore traces is approached, however, the metaxylem extends tangentially from the protoxylem, forming a flattened band. Shortly after this condition is attained there is a decided tendency for the bundles to join and give rise to a sharply defined cylinder of xylem with occasional parenchyma cells. This wood it must be remembered is below the node and not above, and is found nowhere else in the stem. In some of the largest cones a pair of adjacent bundles may fail to fuse, so that a gap occurs which persists until the traces to the first whorl of sporangiophores are freed.

In large well-developed cones gaps occur above the first, second, and third whorl of sporangiophore traces. There appears to be considerable correlation between the size of the cone axis and the number and size of the gaps, for two gaps are common above the first whorl of traces where the axis is large and only one per internode above the second whorl of traces where the axis is noticeably smaller. The position of the gaps for the most part is directly above the traces; but the relation to the trace is apparent rather than real, and is probably due to a general dying out of xylem. In no case does a departing trace leave a break in the xylem.

The sporophore traces alternate with those in the whorls above and below, as do the bundles in the stem. The bundles above the fourth whorl of traces unite, forming a protostele which dies out in

the tip of the protective cap. The endodermis is absent from the traces and disappears in the final internode.

In the small cones the vascular bundles remain united throughout their whole length into either a cylinder (fig. 16) or a solid strand. A few parenchyma cells may occur here and there, but they are so irregular in occurrence that they cannot be regarded as the gaps so common in cones of *Equisetum*. After the departure of the first whorl of sporangiophores, the stele is so reduced in size that only three or four pith cells remain in the center; and finally the position of the pith is occupied by protoxylem and a few parenchyma cells (fig. 17). A further reduction occurs after the second whorl of traces is given off, there being only about a dozen xylem cells remaining as an irregular but unbroken rod of xylem surrounded by phloem (fig. 18). When the third and last whorl of traces is formed, no further differentiation of stelar tissues occurs.

Discussion

The present observations on the differentiation of the segments from the apical cell agree with those of REESS (**23**), CRAMER (**16**), CAMPBELL (**12**), and VIDAL (**35**) in that: the first anticlinal wall divides the segments into a lower and upper cell (the node and internode, respectively), and the first periclinal division cuts off pith on the inside and the parent of stele, cortex, and epidermis on the outside.

The derivation of the pericycle, endodermis, and one or more layers of the inner cortex from the stele is similar to that reported by SCHOUTE (**27**) and BARRATT (**3**) in *Hippuris*, but is contrary to the classical account of VAN TIEGHEM (**33**) to the effect that the endodermis in *Equisetum* is cortical in origin. The majority of recent studies indicate a stelar origin for the endodermis; for instance, CHANG (**13**) has found it thus in *Pteris aquilina*, as has BARCLAY (**2**) in *Selaginella wildenovii;* but BARTOO (**6**) found it to be cortical in *Schizaea pusilla*. In general, EAMES and MACDANIELS (**19**) consider the endodermis to be stelar. In *Equisetum* the situation in the root is entirely reversed, with the endodermis cortical in origin. Furthermore, the work of CONARD (**15**) on *Dennstaedtia punctilobula*, *Cibotium regale*, *Aspidium molle*, *Lygodium japonicum*, *Onoclea sensibilis*,

Ceratopteris thalictroides, and *Aspidium marginale;* that of BARTOO (**5, 6**) on *Schizaea rupestris* and *S. pusilla;* and of STRASBURGER (**31**) on *Lycopodium selago*, seems to be sufficient evidence for regarding the endodermis of the root as cortical throughout the pteridophytes.

The root of *Equisetum* in textbooks and elsewhere is described as having a double endodermis but no pericycle; it is further pointed out that the endodermis is peculiar in that the cells of the inner layer have walls of uniform thickness and therefore lack the characteristic radial thickenings or Casparian strips. STRASBURGER (**30**) has suggested that these two layers are a phloeoterma in which the outer functions as a true endodermis and the inner as a pericycle. Few indeed have followed this suggestion; largely it would seem because at present the terms endodermis and phloeoterma are almost synonymous, and also because of the importance still attached to HANSTEIN's theory of histogens. The evident relationship between the inner and outer endodermis is perfectly clear, for both are composed of the same number of paired cells which are definitely derived from the cortex. To those holding HANSTEIN's theory of histogens, such evidence, together with the fact that there is no other layer which could be designated as pericycle, would be sufficient for regarding the pericycle as absent. The early separation of tissues as implied by the theory of histogens has long since proved unreliable, so that there is no reason why the inner endodermis should not be termed pericycle. On the other hand, as has been pointed out in a previous paper (**21**), the pericyclic nature of the so-called inner endodermis is shown by its position, continuity with the pericycle of the stem, and its ability to produce lateral rootlets as is the case in the spermatophytes. According to our present accounts *Lycopodium* is the only other pteridophyte which shares with *Equisetum* in the production of lateral roots from the pericycle instead of the endodermis.

It is apparent, then, that the root in *Equisetum* is no longer to be considered unique because of its peculiar double endodermis, but rather because its pericycle is cortical and consists of cells similar in general shape and number to the true endodermis. It will doubtless be necessary to extend the change in terminology herein suggested to the root of *Calamites*, in which the so-called double endodermis of

Equisetum occurs. The inner layer here should in all probability be referred to as pericycle.

While the reduction of xylem is not a new observation for *Equisetum*, it is significant that in the internode of both the aerial stem and the rhizome, the tracheids nearest the protoxylem are the ones which fail to mature. This is further evidence in support of the current view that the bundle has been derived by reduction from a unit structure. The most striking reduction though is in the supranodal wood, which at times may be completely absent above the leaf trace, leaving a definite gap in the xylem. Only the presence of a well-defined endodermis prevents one from regarding this as a true foliar gap. Indeed, such a gap in the wood of an angiosperm with a poorly developed endodermis would be considered as foliar in nature without question. No other species of the genus shows such extreme reduction in the supranodal wood; nor is so near an approach to a foliar gap seen elsewhere in the Lycopsida.

In discussing the phylogenetic aspects of the supranodal wood in *Equisetum*, BROWNE (10) suggests that its retention is correlated with the vascular supply of the branches. My observations indicate that a marked relationship exists between size of stem, number of buds, and amount of supranodal wood formed. If the stem is large and has but a single branch, the supranodal wood may be entirely absent above the leaf trace; while if there are two buds it will be well developed above the trace between them. But if the stem is small with either one or two buds, a complete cylinder results. BROWNE'S view is clearly supported and strengthened by this evidence.

Tyloses in vessels, tracheids, and canals formed by the breakdown of protoxylem elements are of common occurrence in both fossil and living plants. STRASBURGER and SYKES make brief mention of them in the carinal canals of the larger species of *Equisetum*, but even there they are seldom found and no one appears to have noted them in the smaller species. Their rarity is attested by the fact that in my observations on a great number of stems they have been seen but twice—once beautifully developed in all four carinal canals of a rhizome, and again rather sparingly at the base of a cone. It is difficult to understand why they occur so infrequently; conditions for their development would seem to be favorable, for the protoxylem

elements are disrupted before the surrounding parenchyma cells show signs of senility. In this connection it is interesting to note that SEWARD (**28**) has found thin parenchyma cells in the carinal canals of *Calamites* which may be interpreted as tyloses.

The position and distribution of the stomata is similar to that in the other species. Their origin and development is as described by STRASBURGER (**29**) for *E. limosum*. The water stomata found on the upper surface of the leaves have not been described before, although WILSON (**38**) in a popular paper on excretion of water figures a specimen of *E. arvense* illustrating guttation. It would seem that hydathodes might be of considerable significance in plants with a limited vascular system; in this instance is may be that they are a determining factor in the retention of the apparently functionless leaves of *E. arvense* and *E. scirpoides*.

It is generally thought that *E. scirpoides* is an unbranched form. CAMPBELL (**12**) expresses this opinion, and adds that the existence of rudimentary buds has not been investigated. Careful examination of plants in the field reveals that most of the aerial stems are really branches from the lower nodes of other stems, and that branching is not uncommon even from the upper nodes. The latter is especially true when the stem tip has been injured. One might therefore expect to find dormant or rudimentary buds in normal stems which my work demonstrates in all stages of development, from initials to completely organized branches with nodes, internodes, leaves, and roots. The close association between branch and root has long been noted but has not received due attention. *Equisetum* is unique in that every branch, whether developed or dormant, is a potential plant equipped for immediate growth, as may easily be shown by allowing stems of one or more nodes to stand in water 7–10 days. Roots and an aerial stem soon appear, and these it must be remembered are not regenerated structures but represent growth in organs previously formed, or that were already in the process of development. Under greenhouse conditions, new plants frequently arise from detached stems which have fallen on the moist soil in the bench. It is likely that this method of vegetative reproduction has had and does have significant survival value in *E. scirpoides* and other species growing naturally in moist habitats.

Considerable discussion is found in the literature as to the origin of the roots in *Equisetum*. All agree that they appear in connection with the branches but the position of the root initials is questioned. JANCZEWSKI (**20**) reported them to be endogenous in *E. arvense* and *E. limosum;* while VAN TIEGHEM (**34**) later found them to be exogenous in *E. palustre*, and designated an outer cell of one of the original segments as the root initial. VIDAL (**35**) came to the conclusion that the root initial was exogenous, although he could not identify it nearly so early as VAN TIEGHEM. A comparison of the figures shown by these writers leaves some doubt as to whether a root initial as figured by VAN TIEGHEM could develop as seen in VIDAL's excellent illustrations. VIDAL's figures, however, appear to be the more convincing of the two. He has drawn the root initial on a line of cleavage between branch and main stem, but the branch is so far advanced that it is impossible to determine with certainty whether the initial is arising from a truly exogenous surface or one which obtains that appearance by a cleavage line between branch and main axis. CAMPBELL (**12**) is of the opinion that the root is endogenous in *E. telmateia*. My observations in *E. arvense* and *E. scirpoides* not only confirm those of JANCZEWSKI and CAMPBELL, but trace the root initial to an inner cell from one of the original segments of the branch. It is clear then that in *E. arvense*, *E. limosum*, *E. scirpoides*, and *E. telmateia* the root is endogenous; while it may well be exogenous as reported in *E. palustre*.

The root tissues are very regular, being almost diagrammatic in their arrangement and development, as has been reported for the other species. The prominently placed central metaxylem cell seen in *E. hyemale*, *E. telmateia*, and others does not occur. Instead the three inner cells from the three large sextants fail to divide, but remain about equal in size although those which mature into metaxylem become slightly enlarged. This shows how the central parenchyma common to the larger roots has been reduced by the failure of these cells to divide.

The anatomy of the cone in *E. scirpoides* differs from that in the larger species in two respects: first, the sporangiophores alternate almost as regularly as the leaves; and second, even though the xylem is much reduced the extensive gaps and meshes due to its dying

out in the large cones are not found. The gaps when present are small and may be entirely absent. This situation is due to the fact that further reduction in xylem would doubtless interfere with the function of the cone, so the amount of xylem remaining is large in proportion to the size of the stele. The structure of the cone shows this to be the case, for as the tip is approached the size of the stele decreases more rapidly than does the number of tracheids. This results in smaller and fewer gaps until they disappear, leaving a siphonostele, while at the extreme tip the xylem is in the form of a solid strand. The formation of gaps, then, is dependent not only upon a reduction of xylem, as has been generally proposed, but upon the failure of the fundamental tissues to reduce in the same proportion. This it would seem is the key to an understanding of the large stelar gaps of the vegetative axis. It is evident that the extent of the meristem and the length of its period of activity before maturation commences determine the size of the stele. In a plant with an apical cell and only primary growth, it is doubtful whether it is possible for the factors of reduction to influence the early activity of the meristem as much as the tissues which mature from it, without inactivating the apical cell and thus stopping all further growth. A stele with extensive gaps is the inevitable result.

Summary

1. The first periclinal division in each segment from the apical cell of the stem separates pith and "primary cortex," which in turn gives rise to stele and cortex.

2. The pericycle, endodermis, and one or two layers of the inner cortex of the stem are stelar in origin.

3. Evidence is presented for regarding the so-called inner endodermis of the root as pericycle.

4. The endodermis and pericycle of the root are of cortical origin.

5. Dormant branches occur as in other species of *Equisetum*. They are exogenous in origin.

6. The first root developed by the branch is endogenous.

7. The stelar parenchyma in the root is much reduced.

8. Hydathodes of the water stomata type occur on the upper surface of the leaves.

9. Rare but well-developed tyloses are found in the carinal canals.

10. Reduction in xylem is extreme, the supranodal wood being frequently absent above the leaf traces, forming a gap in the xylem.

11. The gaps in the xylem of the cone are correlated with the size of the stele and failure of the fundamental tissue to develop.

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EXPLANATION FOR PLATE VIII

FIG. 30.—Longitudinal section through aerial stem showing young branch; ×125.

FIG. 31.—Transverse section of mature stem showing vascular supply to one of two branches; ×255.

FIG. 32.—Transverse section through internode of rhizome showing carinal canals filled with tyloses; ×190.

FIG. 33.—Transverse section through root tip showing origin of endodermis and pericycle from same layer of cells; ×400.

FIG. 34.—Transverse section through central cylinder of triarch root; note inner cells from three large sextants remaining undivided; ×140.

FIG. 35.—Transverse section through diarch root: note stele suspended by cortical cells; ×115.

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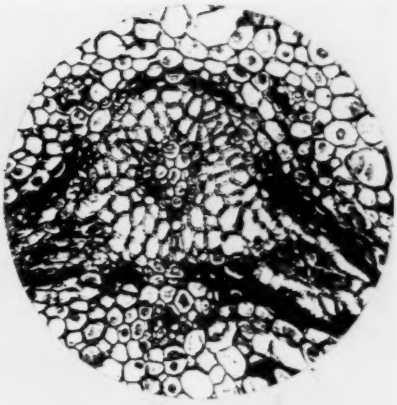

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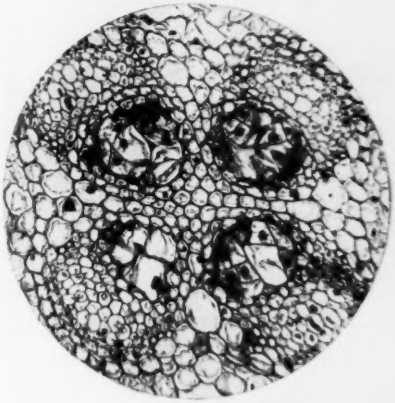

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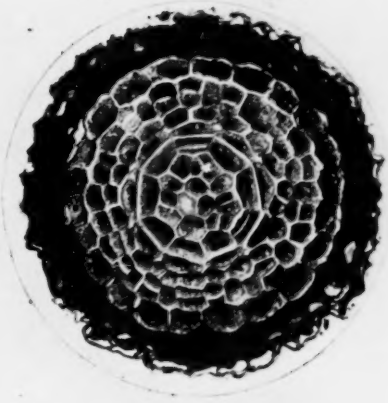

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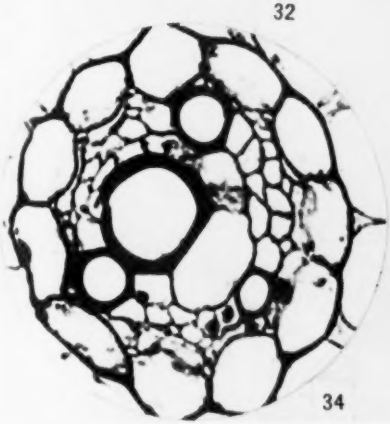

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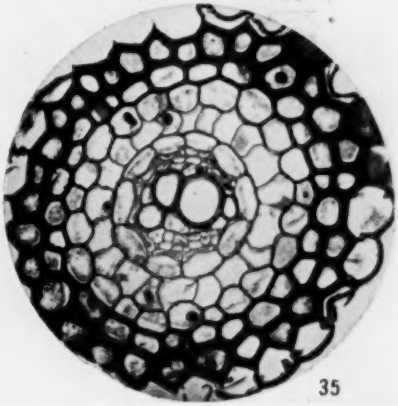

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JOHNSON on EQUISETUM

WALNUT YELLOWS IN RELATION TO ASH COMPOSITION, MANGANESE, IRON, AND OTHER ASH CONSTITUENTS[1]

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(WITH TWO FIGURES)

Introduction

Yellows or little-leaf of walnut trees is a specific chlorotic disease usually considered as being caused by soil conditions rather than having much in common with the diseases known as infectious chloroses. The symptoms of the disease and the nutrition of the trees in health and disease have been discussed by HAAS, BATCHELOR, and THOMAS (**7**). As with pecan rosette which, according to RAND (**14**), has much more in agreement with the infectious type of chlorosis, adequate proof has not been given as yet as to the actual cause. Any studies, including the present, merely constitute additional steps in the study of these baffling tree diseases.

These chlorotic diseases differ somewhat in the different hosts. In mottle-leaf of citrus the disease is at its worst in late autumn and in winter, the spring growth frequently being healthy. In pecan rosette the mottling is described by ORTON and RAND (**12**) and RAND (**14**) as making its appearance toward midseason, with the later-developed leaves presenting the dwarfed, mottled, and roughened appearance typical of the secondary phase. Toward the end of the season, clusters of dwarfed, depauperate branches are usually put out from dormant and adventitious buds farther back on the branches or main trunk. In walnut yellows, on the contrary, the disease frequently is most severe in the first spring growth, often disappearing entirely in the growth of midseason and late season.

Mottled or rosetted citrus, pecan, and walnut trees, when planted in good soil, are known to recover in most cases; conversely, healthy trees, when planted in diseased soil, most frequently show symptoms

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of disease. HAAS, BATCHELOR, and THOMAS (7) found healthy trees of the three species showing their own peculiar type of chlorosis when planted in a guard row of badly mottled citrus that received sodium nitrate, dried blood, and steamed bone-meal as fertilizer. In the field, all or only part of a walnut tree may show symptoms of yellows, and these symptoms may disappear or become more or less severe in succeeding years. In citrus or walnut nursery rows, trees may be affected with disease while adjacent trees but a foot or more away may be healthy, even though the root systems are almost interlocked. Furthermore, healthy walnut trees, grown in large tanks containing soil obtained from holes in the field from which large diseased trees were removed, showed no signs of disease under well-drained soil conditions.

The cause of walnut yellows is not understood. As a result of an investigation of gray-speck disease of oats which proved to be a manganese-deficiency disease, SAMUEL and PIPER (15) recently suggested that possibly pecan rosette, walnut yellows, and mottle-leaf of citrus are manganese-deficiency diseases; and the latter investigator (PIPER 13) has since reported on the limited availability of manganese in certain soils. The suggestion that a manganese deficiency may be the cause of these tree diseases is of interest because of their chlorotic nature and the intimate relation of manganese to chlorophyll formation and destruction.

The writer (6) has recently emphasized the importance of a deficiency or an excess of manganese in the nutrition of citrus. It was shown that when citrus trees were grown in sand cultures with a culture solution containing excessive manganese, the leaves had only certain resemblances to mottled leaves. In the absence of manganese, citrus cuttings in solution cultures showed chlorosis regardless of the amount of iron added to the solution at daily intervals, and in no case could the chlorotic leaves be considered typical mottled leaves. These results with citrus, and the fact that nothing is known regarding the manganese and iron content of walnut trees, make it desirable to investigate the occurrence of these and other elements in walnut trees affected with yellows. The present investigation is a continuation of previous nutrition studies in healthy and diseased walnut trees.

Material and methods

The material used in the present studies consisted of walnut bark and leaves (*Juglans regia*). The bark was cut from the trunks about 3 feet from the ground. In many cases an extremely thin outer layer of bark was first discarded before taking the bark samples. In this way it was hoped to avoid external contaminations.

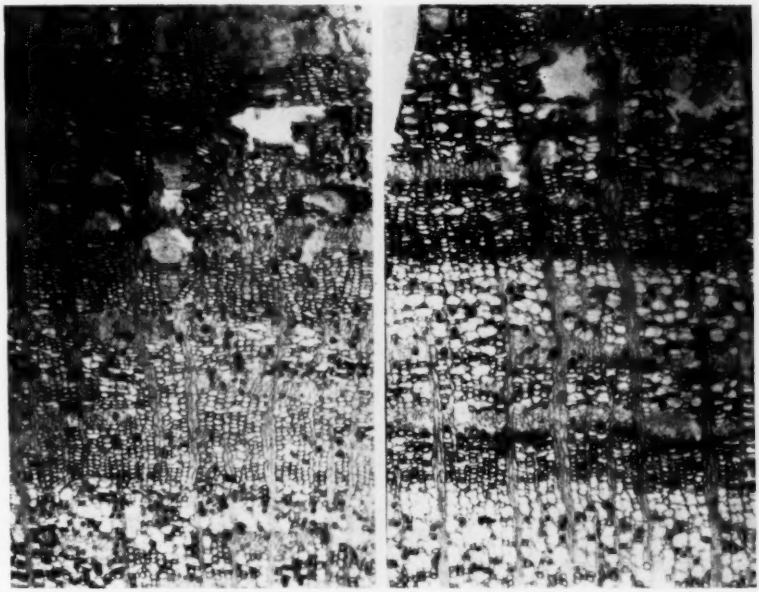

FIG. 1.—Transverse section of walnut tree bark: left, section of healthy tree; right, section of rosetted tree.

The nature of the bark samples used is shown in figure 1. The cells in healthy bark were small and compact, while in diseased bark there was a loose, open structure with many large cells. The bark in both cases contained abundant calcium oxalate crystals.

Care was taken to select only mature leaves for the leaf samples, but even then there was no assurance regarding the average age of the leaves. The results obtained for the leaves, therefore, are not comparable with those obtained by HAAS, BATCHELOR, and THOMAS,

who tagged walnut leaves as they began growth and because of this were assured at least of samples of leaves of similar age.

All samples were dried at 80° C., pulverized in a large porcelain mortar, and dried again at 80° C. Weighed samples were ashed and in most cases determinations were made of the calcium, magnesium, inorganic phosphate, manganese, and iron. Manganese was determined by the periodate method as used by SAMUEL and PIPER, while iron was determined colorimetrically by the method described by ELVEHJEM and HART (4). Calcium was determined by titration with permanganate after double precipitation of the oxalate, while magnesium and inorganic phosphates were determined as pyrophosphates.

Bark

In a preliminary study, walnut trunk bark samples were collected September 27, 1929, from 10-year-old trees at Moreno, California, and on November 2, 1929, from trees of a similar age at Ventura, California. The supply of material obtained was limited. Analysis of the healthy and diseased samples showed the composition as given in table I.

The dry matter of the diseased bark or a water extract contained more ash, calcium, and magnesium than the healthy bark or its water extract. In general the percentages of potassium were greatest in the ash of healthy bark. No definite trend was evident in the percentages of calcium or of sodium in the ash. These results are in agreement with previous studies (7), in which it was found that the composite sap obtained by suction from walnut branches affected with yellows contained more ash and calcium than that obtained from healthy branches.

Another supply of bark material was obtained from 10-year-old trees in a large orchard at Moreno, where a fertilizer experiment conducted by the Department of Orchard Management was in progress. The experimental portion of the orchard consisted of about fifteen rows of twelve trees each. An entire row received a given treatment as regards fertilizer applications. Bark and leaf samples could therefore be obtained from healthy and diseased trees of similar age grown under a given soil management. A thin peel was removed from the samples before they were cut from the trees. The analytical results are shown in table II.

TABLE I

INORGANIC CONSTITUENTS OF HEALTHY AND DISEASED WALNUT BARK (PER CENT)

		VENTURA, CALIF.			MORENO, CALIF.							
		HEALTHY TREE	DISEASED TREE		HEALTHY TREE				DISEASED TREE			
Ash in dry matter	Soluble	1.37	1.99	1.45	1.22	1.35	1.25	0.91*	1.24*	1.53	1.49	1.59
	Total	11.73	12.43	13.26	13.34	12.96	13.10	12.50	15.37	16.59	16.83	15.69
Ca in dry matter	Soluble	0.23	0.39	0.25	0.18	0.22	0.17	0.07	0.12	0.28	0.23	0.29
	Total	4.23	4.42	4.92	5.05	4.79	5.00	4.79	5.43	6.32	6.14	5.93
Ca in ash	Total	36.09	35.56	37.13	37.87	36.95	38.21	38.32	35.32	38.10	36.49	37.83
Mg in dry matter	Soluble	0.13	0.22	0.18	0.066	0.048	0.032	0.016	0.056	0.080	0.082	0.096
	Total	0.28	0.42	0.34	0.16	0.19	0.12	0.08	0.18	0.24	0.19	0.25
K in dry matter	Soluble	0.25	0.28	0.24	0.34	0.32	0.37	0.39	0.35	0.38	0.41	0.37
	Total	0.27	0.30	0.28	0.38	0.36	0.45	0.44	0.40	0.43	0.44	0.39
K in ash	Total	2.30	2.43	2.08	2.85	2.76	3.45	3.52	2.60	2.57	2.60	2.46
Na in dry matter	Soluble	0.04	0.06	0.11	0.12	0.09	0.12	0.08	0.14	0.14	0.11	0.09
	Total	0.09	0.10	0.14	0.16	0.19	.....	.....	.....	0.15	0.16	0.15

* Bark samples from which extreme outer portion was discarded.

TABLE II

COMPOSITION OF BARK OF HEALTHY AND DISEASED WALNUT TREES AS DETERMINED ON SAMPLES COLLECTED AT MORENO, CALIFORNIA

	Treatment and tree condition															
	No soil treatment		Ammonium sulphate 4 lb. per tree		Complete fertilizer 10 lb. per tree		Blood 6 lb. per tree		Manure 10 cu. ft. per tree		Sulphur 10 lb. per tree		No soil treatment		Potassium chloride 8 lb. per tree	
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
	September 25, 1930															
Ash (% in dry matter)	14.55	15.32	11.81	14.65	12.46	16.21	12.99	14.18	11.42	12.64	11.31	12.16	10.61	13.23	12.77	13.98
Ca (% in dry matter)	5.68	6.38	5.02	6.06	5.14	6.78	5.35	5.94	4.74	5.29	4.70	5.07	4.32	5.44	5.32	5.96
Ca (% in ash)	39.18	41.66	42.50	41.34	41.28	41.84	41.20	41.86	41.42	41.84	41.52	41.70	40.76	41.12	41.66	42.64
Mg (% in dry matter)	0.085	0.133	0.070	0.205	0.061	0.165	0.103	0.084	0.085	0.059	0.106	0.124	0.084	0.150	0.076	0.086
Mg (% in ash)	0.59	0.87	0.60	1.40	0.49	1.02	0.80	0.59	0.74	0.47	0.94	1.02	0.80	1.17	0.59	0.62
Mn (p.p.m. in dry matter)	196	200	63	193	107	212	64	50	88	28	64	102	49	150	87	140
	July 15, 1931															
Ash (% in dry matter)	12.68	12.40	12.93	15.57	13.07	13.84	13.77	14.46	12.71	14.72	13.20	13.17	10.74	15.44	12.41	12.55
Ca (% in dry matter)	5.25	5.32	5.52	6.60	5.41	5.70	5.90	6.07	5.45	6.18	5.64	5.68	4.51	6.54	5.23	5.32
Ca (% in ash)	41.40	42.93	42.67	42.38	41.40	41.17	42.86	41.99	42.85	41.98	42.76	43.14	41.96	42.39	42.14	42.28
Mg (% in dry matter)	0.140	0.124	0.116	0.208	0.118	0.188	0.132	0.154	0.088	0.150	0.152	0.096	0.100	0.162	0.174	0.124
Mn (p.p.m. in dry matter)	17	26	31	35	34	50	38	54	18	32	26	42	42	44	24	23
Fe (p.p.m. in dry matter)	869	220	259	240	875	810	300	909	1111	762	839	960	298	896	834	653

* H, healthy; D, diseased.

TABLE II—*Continued*

	Treatment and tree condition													
	Copper sulphate 3 lb. per tree		Pruned; ammonium sulphate 4 lb. per tree		Pruned; no soil treatment		Iron sulphate 3 lb., calcium nitrate 6 lb., ammonium nitrate 3 lb. per tree		No soil treatment					
	H	D	H	D	H	D	H	D	H	D	H	D	H	D
	September 25, 1930													
Ash (% in dry matter)	12.28	12.76	13.47	14.51	12.12	11.67	13.37	12.12	11.04	13.07	13.09	17.18	12.07	15.68
Ca (% in dry matter)	5.05	5.32	5.60	5.99	5.02	4.78	5.56	4.92	4.54	5.40	5.50	7.22	5.01	6.45
Ca (% in ash)	41.14	41.70	41.82	41.28	41.48	41.00	41.60	40.60	41.16	41.34	42.06	42.04	41.54	41.16
Mg (% in dry matter)	0.052	0.096	0.070	0.111	0.065	0.092	0.086	0.089	0.067	0.073	0.060	0.143	0.061	0.18
Mg (% in ash)	0.42	0.75	0.52	0.77	0.53	0.79	0.64	0.74	0.61	0.56	0.46	0.83	0.50	1.11
Mn (p.p.m. in dry matter)	54	63	82	57	52	52	33	60	59	46	48	242	48	106
	July 15, 1931													
Ash (% in dry matter)	12.63	15.09	12.79	15.65	13.02	13.28	11.42	17.26	12.69†	16.47†				
Ca (% in dry matter)	5.45	6.40	5.51	6.58	5.58	5.65	4.78	7.36	5.39	6.93				
Ca (% in ash)	43.17	42.44	43.11	42.01	42.84	42.52	41.86	42.63	42.44	42.08				
Mg (% in dry matter)	0.154	0.128	0.172	0.220	0.136	0.200	0.078	0.158	0.148	0.170				
Mn (p.p.m. in dry matter)	38	66	22	30	20	36	37	53	76	100				
Fe (p.p.m. in dry matter)	851	376	960	872	1473	262	574	1569	328	719				

† No removal of a thin external layer made prior to cutting bark sample from trunk.

The percentages of ash, calcium, and magnesium in the dry matter, and of magnesium in the ash, were higher in diseased than in healthy bark. The percentages of calcium in the ash did not differ greatly, and later analyses also showed that the slight differences were not consistent. It is evident also that the diseased bark contained more manganese than did healthy bark. These results for manganese do not support the suggestion of SAMUEL and PIPER (**15**) that walnut trees affected with yellows may be deficient in manganese. In other papers (**5**, **6**) it was shown by the writer that in mottle-leaf of citrus the trees are not suffering from a deficiency of manganese.

In July, 1931, additional bark samples were obtained from other trees in the tree rows at Moreno, and their analytical results are presented in table II.

The percentages of ash, calcium, magnesium, and manganese in the dry matter of diseased bark were again greater than those of healthy bark. The ash of the unpeeled bark showed more iron in diseased than in healthy bark. The results for the peeled samples were inconclusive.

Additional data are given in table III. It will be seen that most of the bark samples obtained on July 20, 1931, were peeled prior to the cutting of the samples. The few additional manganese determinations all pointed to a greater concentration of manganese in the bark of diseased trees. The unpeeled samples showed more iron and phosphate in diseased than in healthy bark. The results for iron and inorganic phosphate in the peeled bark were not consistent. It should be mentioned that in determining iron in bark samples, considerable difficulty was experienced. In most cases the color obtained with thiocyanate changed so rapidly that it was impossible to secure a satisfactory reading, even when phosphate was first removed as was the case in all iron determinations recorded in this paper. Rapid removal of the color with amyl alcohol did not overcome the difficulties. Qualitative analysis showed that the bark samples were rich in copper, and it was found that small additions of copper salts seriously affected the color obtained with thiocyanate and iron. A special study of the relation of copper to walnut yellows and ash composition is contemplated.

TABLE III

MANGANESE, IRON, AND INORGANIC PHOSPHATE CONTENT OF HEALTHY AND DISEASED WALNUT BARK SAMPLES COLLECTED AT MORENO, CALIFORNIA, JULY 20, 1931

	TREE CONDITION											
	H*	D	H	D	H	D	H	D	H	D	H	D
Manganese (p.p.m. in dry matter)	.....	.....	18	20	45	62	.....	.....	.....	.....	.....	.....
Iron (p.p.m. in dry matter)	792	972	265	536	918	630	889	312	783	366	188	316
Inorganic phosphate (% in dry matter)	0.28	0.20	0.21	0.28	0.45	0.42	0.40	0.30	0.53	0.24	0.19	0.23

TABLE III—*Continued*

	TREE CONDITION											
	H	D	H	D	H	D	H†	D†	H†	D†	H	D
Manganese (p.p.m. in dry matter)	.....	.....	.....	.....	.....	.....	.....	.....	66	104	17	24
Iron (p.p.m. in dry matter)	290	474	637	182	233	294	163	200	137	260	1941	1080
Inorganic phosphate (% in dry matter)	0.25	0.27	0.31	0.33	0.20	0.32	0.20	0.29	0.20	0.33	0.60	0.41

* H, healthy; D, diseased.

† No removal of a thin external layer made prior to cutting bark samples from trunk.

It was mentioned in connection with table III that the unpeeled samples showed more iron and phosphate in diseased than in healthy bark. Accordingly bark samples secured at Moreno on April 20, 1932, were unpeeled except in four samples. It will be seen from table IV that calcium, magnesium, manganese, and inorganic phosphate were generally higher in diseased than in healthy bark. Unpeeled bark usually gave more consistent results than peeled bark. Unpeeled bark samples were thoroughly scrubbed with dry brushes and then wiped clean before the samples were taken, so that contaminations from field sources were at a minimum and no appreciable part of the organic matter of the sample with its ash constituents was lost.

Leaves

Table V gives the results obtained for walnut and a few pecan leaf samples. Even though the samples were collected early in summer and vary somewhat in age, it is clear that manganese was not deficient in either the diseased walnut or pecan leaf samples.

A series of walnut leaf samples were collected at Moreno from the same trees from which the bark samples were obtained, and at the same time. As was pointed out by HAAS, BATCHELOR, and THOMAS (**7**) for walnut leaves and by HAAS and HALMA (**8**) for citrus leaves, the inorganic composition is continually undergoing change. The leaves in the present case were not tagged and were random samples of what were considered mature leaves. The ash and calcium in tables VI and VII showed no obvious relation between healthy and diseased leaves. HAAS, BATCHELOR, and THOMAS, with tagged leaves of equal age, found magnesium to be higher in the diseased leaves. The data in tables VI and VII further confirm that diseased leaves contain a higher inorganic phosphate content than healthy leaves. LOEW (**10**), TRUOG (**16**), and others have pointed out that magnesium increases the absorption and utilization of phosphorus. Although not emphasized by KELLEY and CUMMINS (**9**), their data also show an increased magnesium as well as phosphate content in mottled citrus leaves. Magnesium is a constituent of chlorophyll. It would seem that these mottled citrus or walnut leaves, being yellow between the veins, would have less chlorophyll than would healthy leaves and consequently less magnesium. One must not con-

TABLE IV

CALCIUM, MAGNESIUM, INORGANIC PHOSPHATE, AND MANGANESE IN DRY MATTER OF DISEASED AND HEALTHY BARK SAMPLES COLLECTED AT MORENO, CALIFORNIA, APRIL 20, 1932; SAMPLES NOT PEELED EXCEPT WHERE NOTED

	No soil treatment		Ammonium sulphate 4 lb. per tree		Comflete fertilizer 10 lb. per tree				Blood 6 lb. per tree			Manure 10 cu. ft. per tree		Sulphur 10 lb. per tree		No soil treatment		Potassium chloride 8 lb. per tree	
	H*	D	H	D	H	D	H	D	H	D	D	H	D	H	D	H	D	H	D
Ca (per cent)	5.42	5.91	5.45	5.67	4.83	5.75	5.17	5.90	5.79	5.96	5.64	5.85	5.58	5.44	5.90	4.81	5.76	5.28	6.03
Mg (per cent)	0.173	0.280	0.169	0.247	0.185	0.207	0.276	0.338	0.171	0.221	0.188	0.156	0.227	0.174	0.172	0.171	0.168	0.145	0.163
Mn (p.p.m.)	48	79	52	58	45	127	58	51	146	119	52	84	138	47	51	132	96	97	108
Inorg. Po_4 (p.p.m.)	509	763	560	718	631	636	602	690	632	600	700	467	675	542	727	569	528	572	473

TABLE IV—*Continued*

	Copper sulphate 3 lb. per tree		Pruned; ammonium sulphate 4 lb. per tree		Pruned; no soil treatment		Iron sulphate 3 lb., calcium nitrate 6 lb., ammonium nitrate 3 lb. per tree			No soil treatment							
	H	D	H	D	H	D	H	D	D	H	D	H	D	H†	D†	H†	D†
Ca (per cent)	5.63	5.57	5.54	6.00	5.37	6.22	5.56	5.98	5.99	5.14	6.14	5.09	6.43	5.70	6.50	5.31	5.42
Mg (per cent)	0.184	0.174	0.167	0.216	0.148	0.255	0.160	0.213	0.154	0.160	0.232	0.149	0.226	0.162	0.202	0.156	0.151
Mn (p.p.m.)	62	94	60	146	58	76	64	107	111	92	99	76	132	23	121	39	30
Inorg. Po_4 (p.p.m.)	671	581	662	669	522	633	548	940	448	563	608	687	720	478	670	684	568

* H, healthy; D, diseased.

† Extremely thin layer of outer bark peeled off prior to securing samples.

clude that all magnesium in a leaf is associated with chlorophyll, however, for then the concentration of magnesium in a leaf might be in some degree a measure of the chlorophyll contents.

Tables VI and VII show diseased leaves to have a larger manganese and iron content than healthy leaves. A deficiency of manganese in the diseased leaves did not appear to exist as suggested by SAMUEL and PIPER. There is as yet only a very hazy conception of

TABLE V

ASH, MANGANESE, AND CALCIUM CONTENT OF HEALTHY AND DISEASED WALNUT AND PECAN LEAVES, GROWN IN CALIFORNIA AT LOCATIONS INDICATED

	Walnut leaves										Pecan leaves					
	June 5, 1925		June 22, 1925				July 1, 1925				June 9, 1925		June 22, 1925		June 22, 1925	
	Orange County		Orange County			Riverside	Whittier				Orange County		Orange County		Orange County	
	H*	D	H	D		D	H	D			H	D	H	D	H†	D†
Ash (% in dry matter)	8.38	10.42	11.30	7.19	10.34	9.71	10.95	14.60	10.58		7.68	9.65	9.64	8.89	8.58	9.11
Mn (p.p.m. in dry matter)	92	108	68	50	80	88	44	42	56		144	340	220	240	177	320
Ca (% in dry matter)	1.50	2.01	2.37	1.64	2.01	1.80	2.62	2.88	2.02		1.86	2.16	2.50	1.90	2.18	2.11
Ca (% in ash)	17.90	19.33	21.00	22.86	19.43	18.58	23.97	19.70	19.13		24.23	22.39	25.92	21.38	25.38	23.18

* H, healthy; D, diseased.

† Sample consisted of healthy appearing leaflets of basal half of primary petiole.

what availability of an element within the plant actually means. In the case of iron, some investigators find chemical analysis indicating an excess within chlorotic leaves and still the plant may respond to the further application of iron to the soil or its injection into the tree. This has been attributed to faulty distribution within the plant, non-availability, or insolubility. The writer knows of no such situation in the case of manganese.

There is a distinction between the manganese relation in rosetted and in etiolated leaves. BERTRAND and ROSENBLATT (3) found that etiolated leaves of vegetables contained less manganese than green leaves, while tables VI and VII show that rosetted leaves contained a higher manganese content than healthy leaves.

TABLE VI

COMPOSITION OF MATURE LEAVES OF HEALTHY AND DISEASED WALNUT TREES AS DETERMINED ON SAMPLES COLLECTED AT MORENO, CALIFORNIA, SEPTEMBER 25, 1930

	No soil treatment		Ammonium sulphate 4 lb. per tree		Complete fertilizer 10 lb. per tree		Blood 6 lb. per tree		Manure 10 cu. ft. per tree		Sulphur 10 lb. per tree		No soil treatment		Potassium chloride 8 lb. per tree	
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter)	13.11	15.88	13.78	13.86	14.46	11.74	14.99	15.74	13.58	13.43	11.78	12.26	13.43	13.88	13.19	16.26
Ca (% in dry matter)	3.43	4.31	3.52	3.57	3.62	2.70	3.80	4.07	3.55	3.57	2.86	2.84	3.38	3.42	3.34	4.85
Mg (% in dry matter)	0.54	0.71	0.61	0.80	0.68	0.61	0.65	0.71	0.48	0.61	0.56	0.59	0.52	0.67	0.57	0.53
Mn (p.p.m. in dry matter)	183	253	147	517	217	160	207	240	176	220	168	800	180	328	223	260
Fe (p.p.m. in dry matter)	567	867	800	800	800	400	767	767	633	633	500	767	567	733	717	717
Inorganic Po_4 (% in dry matter)	0.45	0.76	0.51	0.69	0.39	0.55	0.55	0.88	0.47	0.47	0.46	0.57	0.45	0.62	0.57	0.59

TABLE VI—*Continued*

	Copper sulphate 3 lb. per tree		Pruned; ammonium sulphate 4 lb. per tree		Pruned; no soil treatment		Iron sulphate 3 lb., calcium nitrate 6 lb., ammonium nitrate 3 lb. per tree		No soil treatment		No soil treatment		No soil treatment	
	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter)	13.84	16.19	13.30	15.10	15.74	15.23	15.36	13.31	14.94	14.66	14.19	17.37	15.66	13.40
Ca (% in dry matter)	3.74	4.64	3.50	4.06	4.38	4.08	3.88	3.38	3.64	3.36	3.76	5.30	3.84	3.39
Mg (% in dry matter)	0.57	0.77	0.51	0.70	0.64	0.78	0.62	0.61	0.54	0.59	0.56	0.92	0.64	0.76
Mn (p.p.m. in dry matter)	227	483	240	310	210	273	154	350	153	207	180	240	213	358
Fe (p.p.m. in dry matter)	583	717	583	700	700	733	800	800	733	833	666	933	1217	767
Inorganic Po_4 (% in dry matter)	0.51	0.60	0.49	0.94	0.45	0.77	0.56	1.01	0.48	0.68	0.60	0.57	0.59	0.57

* H, healthy; D, diseased.

TABLE VII

COMPOSITION OF MATURE LEAVES OF HEALTHY AND DISEASED WALNUT TREES AS DETERMINED ON SAMPLES COLLECTED AT MORENO, CALIFORNIA, SEPTEMBER 25, 1931

	No soil treatment		Ammonium sulphate 4 lb. per tree		Calcium nitrate 6 lb. per tree		Complete fertilizer 10 lb. per tree		Blood 6 lb. per tree		Manure 10 cu. ft. per tree		Sulphur 10 lb. per tree		No soil treatment	
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter)	14.48	14.55	13.34	14.11	15.34	12.05	12.93	13.30	13.41	13.16	11.58	14.46	12.79	15.40	14.43	14.08
Ca (% in dry matter)	4.13	3.89	3.94	3.96	4.95	3.19	3.93	3.65	4.01	3.59	3.32	4.50	3.62	4.62	4.45	4.11
Mg (% in dry matter)	0.60	0.63	0.41	0.65	0.57	0.47	0.35	0.57	0.43	0.57	0.32	0.50	0.38	0.51	0.43	0.56
Mg (% in ash)	4.14	4.29	3.07	4.61	3.72	3.90	2.67	4.28	3.17	4.33	2.76	3.42	2.97	3.31	2.98	3.94
Mn (p.p.m. in dry matter)	157	236	147	183	133	60	325	433	140	168	113	164	162	258	200	242
Fe (p.p.m. in dry matter)	392	450	233	478	225	367	317	342	291	296	321	417	433	475	225	492
Inorganic Po_4 (% in dry matter)	0.79	0.82	0.70	0.86	0.54	0.88	0.43	0.93	0.42	1.02	0.42	0.59	0.42	0.63	0.49	0.57

TABLE VII—*Continued*

	Potassium chloride 8 lb. per tree		Copper sulphate 3 lb. per tree		Pruned; ammonium sulphate 4 lb. per tree			Pruned, no soil treatment			Iron sulphate 3 lb., calcium nitrate 6 lb., ammonium nitrate 3 lb. per tree		No soil treatment		
	H	D	H	D	H	H	D	H	D	D	H	D	H	D	D
Ash (% in dry matter)	12.63	12.39	13.51	13.77	13.51	12.91	13.26	12.37	14.73	14.80	15.07	12.99	12.65	16.23	15.15
Ca (% in dry matter)	3.96	3.66	4.22	4.15	4.24	3.75	4.03	3.80	4.43	4.33	4.77	3.94	3.81	5.11	4.53
Mg (% in dry matter)	0.54	0.40	0.47	0.58	0.41	0.37	0.68	0.37	0.47	0.45	0.44	0.51	0.34	0.57	0.52
Mg (% in ash)	4.24	3.23	3.44	4.21	3.00	2.87	5.13	2.95	3.16	3.01	2.89	3.89	2.69	3.48	3.43
Mn (p.p.m. in dry matter)	117	183	183	400	83	108	100	83	172	.....	142	164	112	189	178
Fe (p.p.m. in dry matter)	250	217	333	442	383	400	421	358	442	.....	617	817	729	817	917
Inorganic Po_4 (% in dry matter)	0.42	0.56	0.56	0.64	0.44	0.43	0.64	0.41	0.77	.....	0.45	0.61	0.47	0.97	0.79

* H, healthy; D, diseased.

The writer (6), working with citrus cuttings in solution cultures, has found that in most cases less iron was accumulated in the leaves when manganese was made deficient. In rosetted walnut leaves, when manganese was high the iron was also generally higher. McGeorge (11) found a relation between manganese and iron in

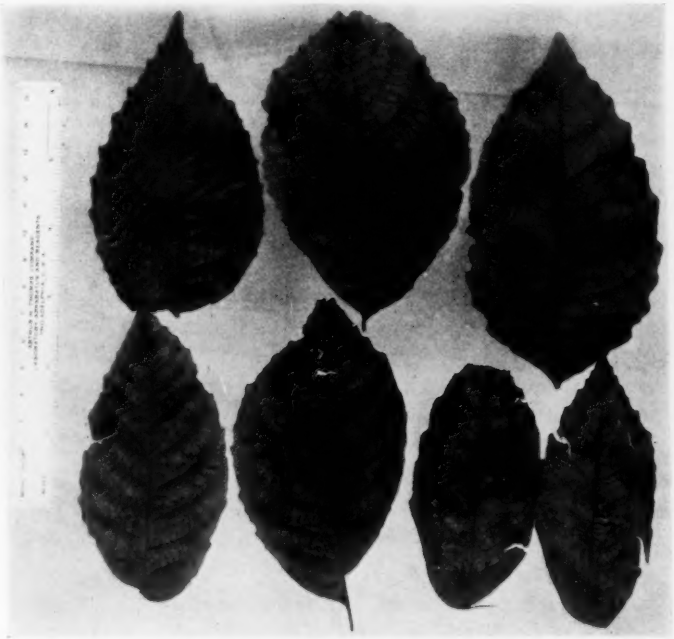

Fig. 2.—Leaflets of walnut seedlings grown in Hoagland's solution containing excessive concentrations of manganese.

sugar cane leaves: less manganese was required if iron was high and less iron if manganese was high.

As a result of dipping or spraying rosetted pecan leaves with ferric sulphate or chloride, Albeen, Cole, and Lewis (1) concluded that pecan rosette is a condition of iron chlorosis. Anderssen (2) has recently found manganese and copper in chlorotic material of deciduous fruit trees to be consistently less than the amount found

* H, healthy; D, diseased.

in healthy material. Manganese applications to the soil were without effect but marked response to copper applications was noted.

An effort was made to grow walnut (*Juglans regia*) seedlings in Hoagland's solution to which were added increasing concentrations (10 to 100 p.p.m.) of manganese as sulphate. When any effect was noted, it usually consisted of either a general yellowing of the entire leaf followed by burning along the veins, or a yellowing between the veins followed by eventual burning of the yellow areas. Figure 2 illustrates the effects of excessive manganese. The effects have some resemblance to those of walnut yellows but are far from being typical of the disease (the control leaf is at the left in upper row). Additional experimentation focused about copper and its interrelations with other elements may throw further light on this baffling disease.

Summary

1. A study was made of certain ash constituents in a large number of samples of healthy and diseased walnut leaves and bark. The ash of diseased bark was higher than that of healthy bark. Calcium, magnesium, manganese, and inorganic phosphate were also generally higher in the diseased bark.

2. Walnut leaves affected with yellows contained a higher magnesium, inorganic phosphate, manganese, and iron content than healthy leaves. Diseased leaves, therefore, cannot be considered as containing insufficient manganese unless a considerable amount of that present is unavailable.

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CYTOLOGICAL STUDIES IN THE GENUS HIERACIUM[1]

ALTON H. GUSTAFSON

(WITH FIFTY-SIX FIGURES)

Introduction

Hybridism is closely related to such phenomena as polyploidy, apomixis, sterility, certain classes of mutations, and a variety of other conditions of general biological interest. ROSENBERG'S (**28**) investigations of a natural *Drosera* hybrid demonstrated a close correlation between meiotic irregularities and the production of sterile pollen. More extended researches by several workers on a number of genera widely separated taxonomically have shown such a close correlation between meiotic irregularities and known and suspected hybrid forms that the presence of such irregularities in a species has come to be regarded as strong evidence for its origin through hybridization.

Hieracium, one of the largest of genera, displays such an intense polymorphism as causes it to be very difficult taxonomically. Hybrids have been recognized in the genus by many observers. OSTENFELD (**23–25**), carrying out numerous hybridization and genetical observations, and ROSENBERG (**27, 29**), investigating the same and similar material cytologically, have demonstrated that hybridization is easily effected in the genus. The cytological work here presented was undertaken in an attempt to add such data as might have a bearing on the important problem of the origin of new forms in nature.

Material and methods

The material, consisting of buds in all stages of development, was collected during the summer months of 1927–1929. Collections were made generally throughout New England and in the provinces of Nova Scotia and Quebec. Herbarium specimens were taken with each collection, in order to check the field determinations. DR.

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

DAHLSTEDT of Stockholm, Sweden, has checked the identifications in the subgenera *Pilosella* and *Euhieracium*.

The buds were picked on warm sunshiny days between the hours of nine in the morning and three in the afternoon. Carnoy's preserving fluid, as recommended by ROSENBERG (**29**), was used for killing. An exhaust pump (JEFFREY **11**) was used to withdraw the air from the tissue to insure rapid fixation. After 24 hours in Carnoy's, the material was washed in several changes of 95 per cent alcohol and transferred to equal parts of glycerin and alcohol in preparation for imbedding. The material was dehydrated and imbedded in nitrocellulose according to the mass method described by JEFFREY (**11**). Sections of 5 and 10 μ were made with a THOMSON-JEFFREY sliding microtome. Haidenhain's iron-alum haematoxylin stain was employed, with a weak solution of eosin in 30 per cent alcohol for cytoplasmic contrast.

Preparations were studied and drawn with the aid of a Bausch and Lomb 1.9 mm., 1.30 N.A., oil-immersion objective and a Leitz 20× periplan compensating ocular. Drawings were outlined with the aid of a camera lucida from typical stages of pollen mother cells during reduction divisions.

Cytological observations

SUBGENUS STENOTHECA

Hieracium venosum L.—ROSENBERG (**27**) first reported the haploid chromosome number of this species as seven, but later (**29**) corrected it to nine. The count of nine was confirmed in the present investigation. The chromosomes differ widely in size but the meiotic processes proceed with absolute regularity. At anthesis the pollen grains are all morphologically perfect.

Hieracium paniculatum L. and *H. scabrum* Michx.—The chromosome number, the meiotic processes, and the pollen conditions are as described for *H. venosum*.

SUBGENUS EUHIERACIUM

Hieracium canadense Michx.—This species is a triploid with 27 chromosomes. Pairing at diakinesis is very irregular. The number of bivalents varies from three to 12.

An equatorial plate is rarely formed. Figure 1 shows a side view of the heterotypic spindle at metaphase with seven bivalents and 13 univalents widely scattered. Figure 2 is a similar view with the entire chromosome complement in the form of univalents widely scattered on the spindle. Polar counts at metaphase vary from 12 to 27. Figure 3 illustrates a cell in which 14 chromosomes are visible. Judging from the size of these, seven are bivalent and seven univalent. Figure 4 depicts an unusual condition in which all 27 chromosomes are visible.

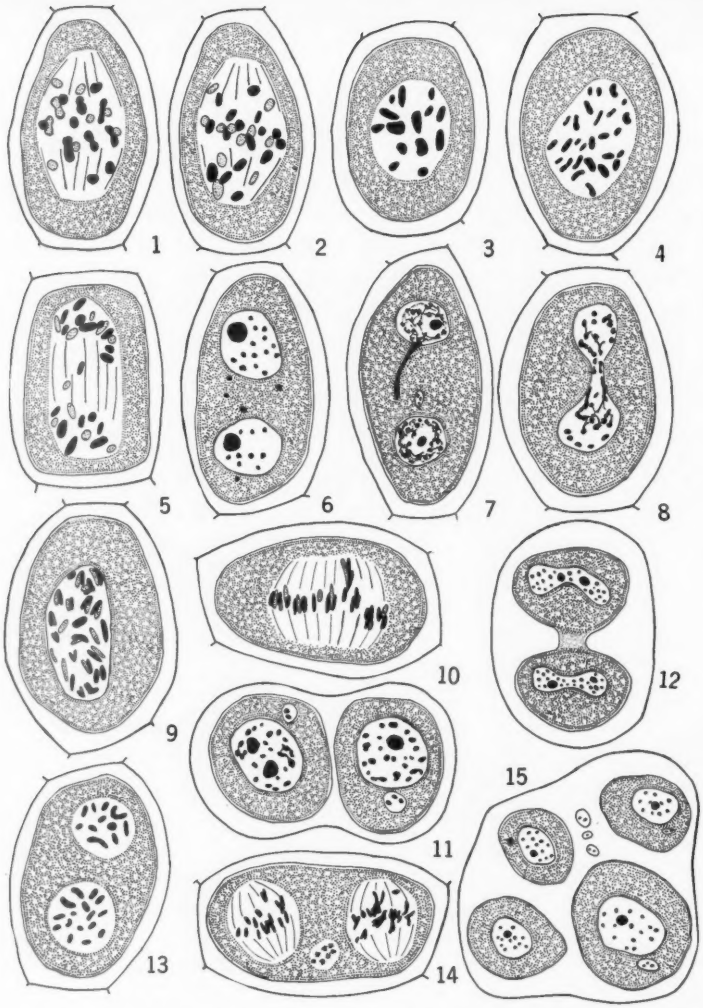
As a result of the irregular metaphase, the distribution of chromosomes to the poles is very unequal. Apparently one member of each bivalent passes to one pole and its partner to the other while the univalents pass to one pole or the other at random. Figure 5 is an anaphase in which 11 chromosomes are found at one pole and 14 at the other, while two are present on the spindle as laggards. As many as 18 have been noted at one pole and as few as seven have been observed. A 13/14 distribution is not uncommon. Lagging is marked. In most cells at least one chromosome and more often several may be seen on the spindle after the majority have reached one pole or the other. The unequal distribution often results in the formation of nuclei considerably different in size. Laggards are frequently extruded into the cytoplasm (fig. 6).

Cells with a number of laggards at anaphase frequently form a "restitution" nucleus which contains the diploid number of chromosomes. Such divisions have been described by ROSENBERG (**29**) and designated as semiheterotypic divisions. Figure 8 represents the dumbbell-shaped nucleus which often results from a division of this type. Figure 7 illustrates a not uncommon condition intermediate between normal telophase and a semiheterotypic division. The nuclear membranes were able to cut through the spindle, but a long "tail" of chromatin almost connects the nuclei, and a micronucleus has been formed from the laggards. In other cases a chain of several micronuclei was observed lying between the two large daughter nuclei. Now and then two nuclei are formed each with an appendage running toward the other. The membranes have cut through the spindle and the only remaining evidence is seen in the misshapen nuclei.

The "restitution" nucleus of the semiheterotypic division undergoes a further division, as seen in the prophase-like condition of figure 9. A number of chromosomes are split lengthwise in preparation for division. It is probable that all the chromosomes divide in this stage. Figure 10 shows the metaphase of such a division. The spindle is extremely broad and the chromosomes form a nearly regular plate. Figure 11 represents a dyad formed as the result of such a division. Probably some laggards are present, for micronuclei have been formed. Many dyads without micronuclei have been observed. Figure 12 represents a condition found in a number of cells. The shape of the nuclei suggests that the original nucleus had divided although the cells had not become completely separated, following which a semiheterotypic division had taken place in each cell of the dyad.

Nuclei such as those shown in figure 6, or those more or less normally formed as a result of the first division, undergo a second division. Since the number of chromosomes in these nuclei varies considerably, the homoeotypic divisions show corresponding variations in numbers. The greater portion of the nuclei pass through a rather regular homoeotypic division without lagging and with an equal distribution of chromosomes to opposite poles. The result of this second division is a cell containing four nuclei, two of which probably differ in chromosome number from the other two, although all the nuclei may contain different numbers as a result of the conditions mentioned. Polar counts of the homoeotypic spindles often show a distribution of 13 and 14. Polar counts show a variation, however, as the 11 in one spindle of figure 13 and the 16 in the other indicate. Although most homoeotypic divisions are rather regular, a considerable number are decidedly irregular. Figure 14 illustrates an irregular metaphase plate and an extruded mass of chromatin lying between the two spindles. The anaphases may show laggards, and the telophase shows that a certain amount of chromatin is often extruded in this division. This accounts for further variation in the chromosome numbers of the nuclei of the tetrad.

Many of the tetrads appear to be in a state of degeneration. The cytoplasm is thin and highly vacuolated, and the cells are irregular in outline. The nuclei vary in size and present a poorly organ-



FIGS. 1.–15.—Pollen mother cells of *H. canadense* during meiosis. Fig. 1, heterotypic metaphase, bivalents and univalents scattered on spindle; fig. 2, heterotypic metaphase, univalents scattered on spindle; fig. 3, heterotypic metaphase, polar view with 14 chromosomes; fig. 4, heterotypic metaphase, polar view with 27 chromosomes; fig. 5, heterotypic anaphase showing lagging and unequal distribution of chromosomes; fig. 6, interkinesis showing extruded chromosomes; fig. 7, interkinesis showing long "tail" of chromatin and a micronucleus; fig. 8, dumbbell-shaped restitution nucleus; fig. 9, restitution nucleus preparing for division, chromosomes splitting; fig. 10, broad metaphase plate formed as restitution nucleus divides; fig. 11, dyad with micronuclei; fig. 12, dyad with restitution nucleus in each cell; fig. 13, homoeotypic metaphases, polar view showing unequal chromosome numbers; fig. 14, homoeotypic metaphases, irregular plates and extrusions in cytoplasm; fig. 15, polycaric and polysporic tetrad. ×970.

ized appearance. It seems that a certain number of potential pollen grains never get beyond the tetrad state.

Polycary and polyspory are frequent features of the later stages of development. Figure 15 illustrates conditions often seen. There are four large cells, no two of equal size, as well as three very small cells. Two of the four large cells contain micronuclei in addition to the normal one, and a third cell shows a small mass of chromatin in the cytoplasm. After the tetrads break up, the young grains often contain one or two small nuclei in addition to the much larger normal one.

These abnormalities indicate that the pollen will present unusual conditions, a high percentage of it being morphologically sterile. Many crushed and empty grains and many microcytes are found in the anthers, together with large grains probably formed from the dyads. It is apparent from the descriptions of the meiotic divisions that the grains may contain chromosome numbers which vary over a considerable range. There are present at anthesis a number of grains which are of normal size, and a considerable number of large grains all apparently sound from the morphological standpoint. No binucleated grains have been found at anthesis, however, although this is the normal condition in species with regular meiotic processes.

The embryos develop apomictically. It is hoped to report upon this more fully at a later date.

Hieracium murorum L.—Herbarium specimens showed the collections falling clearly in the *H. murorum* group but not identical with *H. murorum* of Miss TERRY as reported in GRAY'S manual. Dr. DAHLSTEDT pronounced it *H. murorum* (species near *H. praecox*).

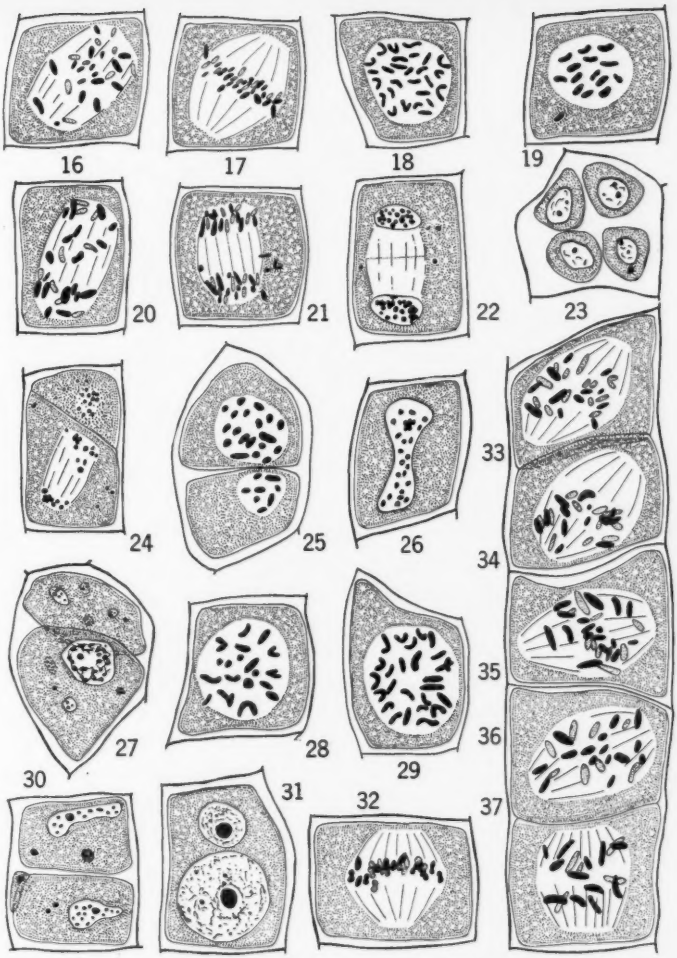
During the prophase there is never a pairing of the chromosomes as is characteristic of the species with regular meiosis. The single chromosomes pass directly from an unpaired condition to the metaphase. Figure 16 represents the usual condition at metaphase, a condition seen in hundreds of cells. The univalent chromosomes are widely scattered on the spindle with no suggestion of the formation of an equatorial plate. Figure 17 illustrates a condition occasionally found. The chromosomes form a regular plate, but more than the expected 27 appear. Apparently some of the univalents have already divided. As might be judged from the preceding figures, counts in

polar view vary greatly. Figure 18 shows a condition observed now and then in which the entire 27 chromosomes are seen. Figure 19 is a similar view in which 14 chromosomes are found in the spindle area and a fifteenth has been extruded into the cytoplasm. Counts ranged from 12 to 27.

The anaphase is also irregular. The foregoing conditions indicate that distribution to the poles is unequal. Figure 20 shows seven chromosomes at one pole and 13 at the other, while seven lie between the poles as laggards. Counts range from seven at one pole and 20 at the other to a 13/14 distribution. Often chromosomes are extruded into the cytoplasm (fig. 21). Interkinesis is illustrated in figure 22. Extruded chromosomes are to be noted in the cytoplasm and a cell plate has been formed; this latter is not formed in species with normal meiosis. Dyads may be formed as a result of the first division, and usually are irregular in some respect. Figure 27 shows one in which a large nucleus has been formed in one cell, together with two small nuclei; and two extruded masses of chromatin are also present. The other and smaller cell contains four micronuclei and a small bit of chromatin. Sometimes no cell plate is formed after the first division, but mostly in such cases the nuclei formed appear to be poorly organized and the cytoplasm is often thin and highly vacuolated.

In many instances a second division follows the first. Figure 24 shows the anaphase of a homoeotypic division in which irregular distribution of the chromosomes, lagging, and extrusion are evident. Figure 25 depicts a polar view of the homoeotypic metaphases. In the lower cell only eight chromosomes are seen, while in the upper 19 are present. The tetrad resulting from the homoeotypic division is shown in figure 23. The cytoplasm is thin and the nuclei are poorly organized. One cell contains a bit of extruded chromatin. Figure 26 illustrates a condition referred to under *H. canadense* as a restitution nucleus. This nucleus rounds up to a normal shape and apparently undergoes division, for several instances were noted in which the chromosomes had apparently divided, as seen in *H. canadense;* but the later stages of division were not seen.

It is curious that in many cells the spindle lies with its axis parallel to the diagonal of the cell rather than parallel to the length, and the



FIGS. 16–27.—Pollen mother cells of *Hieracium murorum* during meiosis. Fig. 16, heterotypic metaphase, univalents scattered over entire spindle; fig. 17, heterotypic metaphase, unusual equatorial plate; fig. 18, heterotypic metaphase in polar view with 27 chromosomes; fig. 19, heterotypic metaphase in polar view with 14 chromosomes on spindle and one extruded in cytoplasm; fig. 20, heterotypic anaphase, irregular distribution of chromosomes and lagging; fig. 21, heterotypic anaphase, extrusion and lagging; fig. 22, interkinesis, cell plate forming, and extrusions; fig. 23, tetrad; fig. 24, homoeotypic anaphases, unequal distribution of chromosomes, and extrusions in cytoplasm; fig. 25, restitution nucleus; fig. 26, homoeotypic metaphases in polar view showing unequal distribution of chromosomes in cells; fig. 27, abnormal dyad.

FIGS. 28–37.—Pollen mother cells of *H. smolandicum* during meiosis. Fig. 28, heterotypic metaphase in polar view with 19 chromosomes; fig. 29, heterotypic metaphase in polar view with 26 chromosomes; fig. 30, dyad with misshapen nucleus, extruded chromatin, small "extra" cell formed; fig. 31, dyad with extreme difference in nuclear size; fig. 32, heterotypic metaphase, equatorial plate formed with dividing univalents; figs. 33–37, cells side by side in same anther; figs. 33–36, heterotypic metaphases with chromosomes widely scattered on spindle; fig. 37, anaphase with portion of cell cut away. ×970.

spindles are often eccentric. Whatever course the divisions may take after interkinesis, the end result is the same in all cases. Dyads, tetrads, or the other varied products of the irregular divisions all degenerate. At anthesis all traces of cell structure have disappeared and the anthers contain only a few bits of entirely degenerate cell fragments. No pollen is formed.

Hieracium smolandicum.—Material was collected along the Big Baddeck River, Nova Scotia, by Professor JEFFREY. Dr. DAHLSTEDT states that it differs from *H. smolandicum* but seems related to that species. A comparison with specimens of *H. smolandicum* subspecies *robinsonii* Zahn in the Gray Herbarium collection makes it rather certain that the specimens are of the same species as discussed here.

The pollen mother cells are similar to those of *H. murorum*, and the same general meiotic irregularities characterize the species. The diploid chromosome number is 27. No pairing of the chromosomes is found at prophase, and the chromosomes appear on the spindle as univalents. Figures 33–37 are especially interesting, for they represent a row of five adjacent cells in the same anther undergoing meiotic division. It is apparent that the chromosomes are all univalents and form no equatorial plate, for they are widely scattered on the spindle. Figure 32 illustrates a condition seen occasionally. The chromosomes form a somewhat regular plate and some of the univalents appear to be dividing. As expected, the polar views vary widely in counts. Figure 28 shows such a view with 19 chromosomes and figure 29 one with 26. Counts ranged from 12 to 26.

As in the case of *H. murorum*, two cells are usually formed after the first division. The dyads produced are generally abnormal (fig. 30). Each cell contains a misshapen nucleus and extruded chromatin. A small bit of cytoplasm containing chromatin has been cut off also. The nuclei are often of very unequal size and micronuclei are usually present. Two nuclei may be formed with no division of the cytoplasm. They are usually unequal in size (fig. 31).

Division figures with all the characteristics of somatic divisions have been observed. The occurrence of a second division has not been observed. No pollen is formed. It is hoped to report upon the apomictic development of the embryos at a later date.

SUBGENUS PILOSELLA

Hieracium flagellare Willd.—Herbarium specimens and collecting bottles were labeled *H. pilosella* var. *viride* at the time of collection. A comparison with specimens at the Gray Herbarium showed similar material had been given the same name, although ZAHN's descriptions indicated that the material was probably *H. flagellare*. Dr. DAHLSTEDT pronounced the material to be *H. flagellare* Willd.

ROSENBERG (**29**) pays little attention to the pollen formation of *H. flagellare*, but does state that the "pollen formation is typical," with 21 bivalents present. If the term "typical" means normal, the following observations hardly bear out this statement; if it means typical of apomictic species in general, the observations confirm the statement.

The spherical mother cells are surrounded by a heavy callose coat. The great number of chromosomes makes a count at diakinesis of uncertain value. Most of the chromosomes are paired although univalents are also found. Side views of the metaphases reveal irregularities. Figure 38 illustrates such a view, in which the bivalents form a plate which is too compact to allow a certain count to be made. Six univalents are found on various parts of the spindle, and two other constricted chromosomes which appear to be rather large for univalents are found on the spindle also. Univalent laggards vary from one to nine. Usually from 10 to 12 bivalents may be distinguished readily. Figure 39 shows an early metaphase in side view with both univalents and bivalents present. Polar views show counts ranging from 18 to 23. Figure 40 illustrates such a view, in which, judging by size, 14 chromosomes are bivalents and four are univalents. Other counts range from 12 to 15 bivalents and from four to nine univalents.

Heterotypic metaphases are seen in figures 41 and 42. The former shows both bivalent and univalent laggards and extrusion; the latter shows laggards of both kinds, and the univalents appear to be dividing. Figure 43 shows the result of lagging. Several chromosomes have not been included in the daughter nuclei. About 10 per cent of the cells show extrusions in this stage. Occasionally, in the anaphase, a long dark streak is to be noted passing from a split chromosome at one pole to another at the opposite pole, just as though a

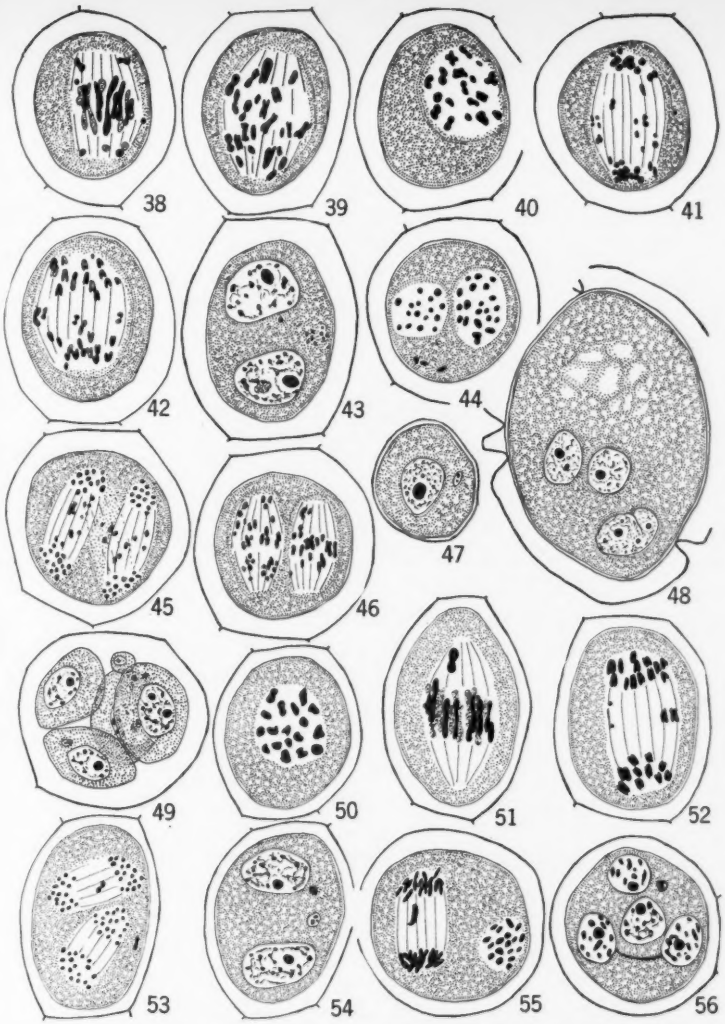
trail of chromatin had been left between the separated members of a bivalent chromosome.

The homoeotypic divisions are also irregular. Figure 46 shows pronounced lagging with chromosomes widely scattered on the spindle. Many cases of normal behavior, or with only one or two chromosomes behaving abnormally, have been observed. The anaphase in figure 45 is very irregular. The center of the cell contains the apparent remains of the heterotypic spindle upon which several extruded chromosomes remain. On each side of this spindle the homoeotypic spindles appear with numerous laggards present. Lagging is more pronounced in the homoeotypic divisions than in the heterotypic. Polar views of the homoeotypic divisions show the variations in numbers which the side views would indicate. Figure 44 shows one spindle with 16 chromosomes while the other has 20, and three large chromosomes are seen extruded in the cytoplasm.

Polycary is commonly noted in the tetrads. The nuclei doubtless contain different numbers of chromosomes, both because of extrusion and because of the presence of both univalent and bivalent chromosomes in the early stages of meiosis. The unequal sizes of the nuclei point to the same conclusion. Polyspory is less frequent. Figure 49 illustrates both polycary and polyspory in the same tetrad.

The pollen grains show great variation in size. A few grains appear empty or degenerate, but the majority, in spite of size differences, appear to be perfect morphologically. Figure 47 shows a young microspore before the tetrad has broken up. The cell is seen to contain an "extra" nucleus. This is a common condition resulting from polycary. Although pollen grains in division show about 20 chromosomes, it has not been possible to establish the chromosome number with accuracy. In a great many cases "giant" grains are formed. Figure 48 illustrates such a grain. In this case three nuclei may be seen, and the lower one is lobed and much larger than the other two. Often the grains are lobulated and decidedly irregular in shape. Cross walls may separate the whole structure into two, three, or four cavities. Judging by the size, shape, and variations in wall structure, these grains have developed from an entire tetrad.

ROSENBERG (**29**) reports the chromosome number of *H. flagellare* as 42. The exact number was not ascertained with certainty in this



FIGS. 38–49.—Pollen mother cells of *H. flagellare* during meiosis. Fig. 38, heterotypic metaphase, bivalents in plate, univalents scattered on spindle; fig. 39, heterotypic metaphase, univalents and bivalents widely scattered on spindle; fig. 40, heterotypic metaphase, polar view of plate with 18 chromosomes (14 bivalent and 4 univalent); fig. 41, heterotypic anaphase, lagging, extrusion, division of univalents; fig. 42, heterotypic anaphase, lagging, division of univalents; fig. 43, interkinesis with several extrusions; fig. 44, homoeotypic metaphases polar view with unequal distribution of chromosomes, extrusions; fig. 45, homoeotypic anaphases, chromosomes widely scattered on spindles; fig. 46, homoeotypic anaphases, irregular distribution of chromosomes, lagging, extrusion, remains of heterotypic spindle; fig. 47, microspore with "extra" nucleus; fig. 48, giant pollen grain formed from whole mother cell; fig. 49, tetrad showing extrusion, polycary, and polyspory.

FIGS. 50–56.—Pollen mother cells of *H. pratense* during meiosis. Fig. 50, heterotypic metaphase in polar view with 18 chromosomes; fig. 51, heterotypic metaphase with regular plate of bivalent chromosomes, single univalent laggard; fig. 52, heterotypic anaphase, two bivalent laggards and split univalent laggard; fig. 53, interkinesis with extrusion and micronucleus; fig. 54, homoeotypic anaphases, lagging, extrusion, and unequal distribution of chromosomes; fig. 55, homoeotypic anaphases, spindle at left showing only one pole, spindle at right with laggard; fig. 56, tetrad showing polycary and band of chromatin connecting two nuclei. ×970.

study, but there are at least 36 and probably not more than 42 chromosomes.

Aposporic embryo sacs reported by ROSENBERG (**29**) have been found. Details of their formation will be given later.

Hieracium pratense Tausch.—The pollen mother cells are much as in *H. flagellare*. At diakinesis the chromosomes are distinctly in pairs, although it is not certain that all of them are in this condition. Heterotypic metaphases in polar view usually show 18 chromosomes (fig. 50). Side views of the plate show the chromosomes arranged regularly. About 10 per cent of the cells in this stage show minor irregularities. Figure 51 is regular except for the bivalent laggard at the upper end of the spindle. A single univalent laggard has been seen in some cells. Polar views sometimes show only 17 chromosomes, as would be expected.

Although the anaphases are mostly regular, irregularities do occur (fig. 52) which show several laggards between the groups at the poles. The cell in this illustration has been partly cut away and does not show the full chromosome complement. The chromosomes are clearly split in preparation for the homoeotypic divisions. At the left are two small chromosomes, probably the result of a split univalent. Interkinesis as seen in figure 54 shows the result of lagging. Between the daughter nuclei a micronucleus and a bit of chromatin are seen. Where divisions are normal, of course, as is the rule in this species, the two daughter nuclei are regularly formed.

The homoeotypic divisions are also normal for the most part. Figure 53 shows a case with minor irregularities. The upper spindle has 17 chromosomes at one pole and 18 at the other, while the lower spindle shows 17 and 19 chromosomes with an extrusion at the right of the figure. Figure 55 shows a single laggard at anaphase.

The tetrads are ordinarily regular, but the slight irregularities already referred to indicate that this is not always the case. Figure 56 illustrates a polycaric condition in which one micronucleus is present in addition to the four nuclei expected. At the lower side of the figure a strand of chromatin connects two of the nuclei. A similar strand was observed in several tetrads.

The pollen grains are morphologically perfect in about 95 per cent of the cases, although a few empty grains are to be found. The

grains are practically the same size throughout, although the abnormalities referred to suggest that some grains may contain 17 or 19 chromosomes instead of the true haploid number of 18.

Apomictic development has been noted and will be reported upon more fully later.

Discussion

Formation of polyploid gametes

The writer's investigation, as well as those discussed here, shows that through abnormal meiotic behavior pollen grains are often produced which vary markedly from the expected haploid chromosome number. These may be designated as polyploid grains, and the gametes which are formed may be called polyploid also. Such grains may be formed in several ways, as follows.

Irregular distribution of chromosomes.—The failure of some or all of the chromosomes to pair at diakinesis leads to the presence of either univalent chromosomes or of both univalent and bivalent chromosomes on the spindle. Under such conditions the equatorial plates may be very irregular, with the chromosomes widely scattered on the spindle. Lagging is often prevalent at anaphase. The varying number of bivalents and univalents and the lagging result in an unequal distribution to the poles. The variation in number is increased by the fact of extrusion. Similar irregularities in the homoeotypic divisions add to the possibilities of an irregular distribution of chromosomes to the daughter nuclei. Polycary, polyspory, and the varying size of the pollen grains indicate that the variation in chromosome numbers may be wide.

Hicks (**10**) in the Cyperaceae, Woodworth (**35**) in *Betula*, and Church (**5**) in the Gramineae support this conception with considerable evidence.

Non-reduction.—Woodworth (**35**) described non-reduction in *Betula*. Rosenberg (**29**) observed this type of division in *Hieracium pseudoillyricum*, and commented upon the somatic appearance of the chromosomes. Each cell of the dyad produced contains the somatic number of chromosomes for the species. *H. murorum* and *H. smolandicum*, as already shown, carry out this type of division. A summary of these investigations indicates that pollen grains with the

somatic number of chromosomes may be produced in known and suspected hybrids by a process of non-reduction.

GIANT POLLEN GRAINS.—Giant pollen grains have been found in several species in widely separated taxonomic groups. CANNON (3), investigating hybrid cotton, ROGERS (26) and CASTETTER (4), studying *Melilotus alba*, TAYLOR (34) in *Acer rubrum*, GAINES and AASE (8) in *Triticum*, and WOODWORTH (35) in *Betula*, have reported the formation of such grains. In each case hybridization is believed to have been responsible for this abnormal condition. MATSUDA (18) found that forms of *Petunia*, probably of hybrid origin, produced giant pollen grains which germinated in artificial culture. The anthers of *Hieracium flagellare* contain a considerable proportion of giant grains apparently developed from entire mother cells. It is clear that giant grains have been observed in a number of cases in known and suspected hybrid forms and that such grains may be viable.

SEMIHETEROTYPIC DIVISION.—ROSENBERG (29) described and figured some unusual departures from the normal meiotic process in *Hieracium*. The heterotypic phase showed no normal plates, but the chromosomes were widely scattered on the spindle. At a stage corresponding to anaphase, the chromosomes were still widely scattered on the spindle as laggards. These were so numerous in many instances that the nuclear membranes normally laid down at the poles, instead of cutting through the spindle figure, inclosed the entire chromosome complement in a single nuclear membrane. The single nucleus thus formed was often dumbbell-shaped and gave the appearance of an amitotic division. The constriction was carried even farther in some cells. In many instances, however, the nucleus was rounded out to a normal shape although it contained the somatic number of chromosomes. Such nuclei correspond to interkinetic nuclei, and have become known as restitution nuclei. Those which are of normal shape undergo a division; each chromosome splits longitudinally and the following division is regular, resulting in a dyad of nuclei each of which is diploid for the species concerned.

A number of earlier cytologists have described and figured abnormal meiotic divisions which were probably semiheterotypic, although not recognized by them as such. More recent investigators,

such as BUXTON and NEWTON (**2**) in *Digitalis* hybrids, KARPECHENKO (**13**) in *Raphanus*×*Brassica*, and WOODWORTH (**35**) in the hybrid *Betula sandbergi*, have recognized this type of division, have described it much as ROSENBERG did, and have applied the term semiheterotypic to it.

The semiheterotypic division is widespread in its occurrence in known and suspected hybrids. It results in polyploid pollen grains which are usually diploid for the species. Slight variations in the process, as described under *Hieracium canadense*, show that in some cases aneuploid dyads are produced.

Polyploid pollen grains may be formed in a number of ways through abnormalities in meiosis. Partial or non-pairing at diakinesis, the presence of both bivalent and univalent chromosomes, lagging, and extrusion, all result in the irregular distribution of chromosomes to the daughter nuclei, so that pollen grains may contain chromosome numbers which vary considerably from the haploid number. Non-reduction, the formation of giant grains, and the semiheterotypic division are other means by which pollen grains containing an abnormal number of chromosomes may be formed. Such grains usually contain the higher polyploid series of numbers.

ORIGIN OF POLYPLOIDY

If it can be demonstrated that polyploid pollen grains do produce viable gametes, a reasonable explanation for the origin of polyploid forms is at hand. Gametes with unlike chromosome numbers may be produced by plants of the same species, or even by the same plant, and these may unite to form polyploid zygotes. Similarly, species of a polyploid series produce gametes with unlike chromosome numbers, and these may unite through interspecific hybridization and produce forms with chromosome numbers differing from those of the parents.

OSTENFELD (**23–25**) investigated *Hieracium* experimentally, and ROSENBERG (**29**) examined the resulting hybrids. Referring to hybrids between tetraploid species, themselves regarded as being of hybrid origin, ROSENBERG says, "Researches on the hybrids concerned have shown that, in fact, the varied chromosome number has not influenced the ability to germinate." Hybrids were also pro-

duced between diploid and tetraploid species (the latter probably hybrid). The F_1 generation showed plants exhibiting an astonishing heterogeneity and displaying varied chromosome numbers. Polyploid gametes are thus seen to be viable and to produce types which may form the basis for a new species.

LJÜNGDAHL (**15**) produced a stable hexaploid type in *Papaver* by crossing *P. striatocarpum* with *P. nudicaule*. The former has a haploid chromosome number of 36 and the latter one of seven. The meiotic divisions of the hybrid exhibited 21 bivalents. Two forms with a tremendous difference in chromosome numbers have been hybridized to form a type with a chromosome number differing from that of either parent. Gametes with widely differing chromosome numbers are thus capable of producing a functional zygote.

The investigations of KARPECHENKO (**12, 13**) on *Raphanus* × *Brassica* yielded significant results. Irregularities of meiosis in the hybrid caused the formation of polyploid gametes with from six to 12 chromosomes, or, by a semiheterotypic division, diploid, hypodiploid, and hyperdiploid and tetraploid cells were formed. The F_2 generation produced tetraploid, hypotetraploid, and hypohexaploid plants. The F_1 back-crossed to *Raphanus* yielded triploid, pentaploid, and hypopentaploid forms. KARPECHENKO says, "Here we have evidently to deal with only progeny of gametes with nine, 18, and 36 chromosomes or of such approaching the above mentioned ones in number of chromosomes." The hybridization of two species with like chromosome numbers has led to irregularities in meiosis, resulting in the formation of polyploid gametes which have been shown to be viable in various combinations and to have produced plants with a wide range of chromosome numbers. Referring to the tetraploids KARPECHENKO says, "They distinguish themselves by quite normal development, by regular meiosis; they have normal pollen and most of them are fertile. After several generations they will probably all be fertile, as progeny from the fertile plants will increase more rapidly than progeny from plants of reduced fertility, and will displace the latter. When comparing it with the parent species *Raphanus sativus* and *Brassica oleracea*, tetraploids represent a different chromosome number. They appear most difficult to cross with *Raphanus* or *Brassica*, i.e., we observe in them the occurrence of the

sexual individualization to which BATESON (1914, 1922) attached special importance for determining a 'specific nature.' "

The *Crepis* hybrids of COLLINS and MANN (**6**) have been extensively investigated. *Crepis setosa* with a haploid number of four and *C. biennis* with a haploid number of 20 were crossed. The F_1 meiotic divisions showed 10 bivalents to be present ordinarily. These probably represent pairings of the 20 *biennis* chromosomes, as *setosa* is shown in other cases not to exhibit pairing among its chromosomes. The gametes contained from 10 to 14 chromosomes. The F_1 individuals back-crossed to *biennis* were found to contain 32 chromosomes. The reduction divisions showed 15 bivalents to be present and reduction was almost normal. COLLINS and MANN consider an F_4 plant sufficiently distinct to call it *C. artificialis*. *Crepis* contains a long series of aneuploid species which they believe to have arisen through hybridization.

Experimental work on the production of polyploids has been carried out by NEWTON and DARLINGTON (**21**). Triploid plants of *Tulipa* have been produced by hybridization of distinct diploid and tetraploid species, as well as by diploid and tetraploid individuals of the same species. Investigation of *Hyacinthus* (NEWTON and DARLINGTON **21**) has led to the following statement: "Perhaps correlated with the ordinary fertility of these aneuploid hyacinths is the fact that hyacinths appear to be equally successful with every chromosome number between 16 and 30, so that gametes with very irregular chromosome numbers are no less likely to produce viable zygotes."

NAWASHIN (**19, 20**) demonstrated the viability of gametes with chromosome numbers other than the haploid number. Triploids of the ordinary *Crepis capillaris*, *C. dioscorides*, and *C. tectorum* appeared in his cultures. The origin is unknown. Seed produced by the open pollination of *C. capillaris* yielded 105 plants. Open pollination of the F_1 gave an F_2 containing every chromosome number from diploid to tetraploid, and in addition a heptaploid plant was found. The triploid plants of *C. capillaris* used as female were crossed with normal plants of various species of *Crepis*. Most of these hybrids showed a diploid complex from *capillaris* and a haploid complex from the other species. Hybrid offspring were also obtained from crosses which had never been successful with diploid *capillaris*.

NAWASHIN states, "these polyploid hybrids of various kinds proved to be much more fertile than the normal diploid ones."

In addition to such experimental studies, there have been many cytological studies on polyploid genera without the background of closely correlated experimental work. Such investigations have had, in many cases, a considerable amount of taxonomic or genetical evidence in support of the cytological results. Among studies of this type are those of TÄCKHOLM (**33**) on *Rosa;* LONGLEY (**16, 17**) on *Rubus* and *Crataegus;* AFZELIUS (**1**) on *Senecio:* HÅKANSON (**9**) and HICKS (**10**) on *Scirpus;* and WOODWORTH (**35**) on *Betula.* These investigators have expressed the opinion that the polyploid series of the investigated genera have arisen through hybridization.

The results of the controlled experimental work and of an examination of natural polyploid series lead to the same conclusions. Polyploid gametes have been produced as a result of hybridization. These gametes have proved viable in various combinations and have resulted in the formation of polyploid hybrid forms. These hybrids intercrossed or back-crossed have led to the establishment of stable types. Such stable types exhibit a different chromosome number from either of the parents and the reduction divisions are regular or nearly so. Certain of these polyploid forms are more fertile than the diploid forms. Some of the types produced have been sufficiently different from any known form to cause a new specific name to be applied to them. The conclusion has been reached that both aneuploid and euploid forms arise through hybridization.

In view of this situation, the statement by DAVIS (**7**) seems particularly appropriate: "There is coming to be recognized a type of hybrid that reproduces itself perfectly, throwing at most only occasional variants. Such hybrids satisfy fully our concept of species as a kind of animal or plant which breeds true."

Summary

1. Cytological examination of the male meiotic divisions of *Hieracium* yields the following results:

a. *Hieracium venosum, H. paniculatum,* and *H. scabrum* of the subgenus *Stenotheca* are characterized by a diploid chromosome number of 18 and exhibit perfectly normal reduction divisions.

b. *Hieracium canadense*, *H. murorum*, and *H. smolandicum* of the subgenus *Euhieracium* are triploid species. Various degrees and combinations of non-pairing, lagging, extrusion, semiheterotypic divisions, polycary, polyspory, and pollen sterility are found in these species.

c. *Hieracium pratense* of the subgenus *Pilosella* is a tetraploid species and reveals lagging and extrusion in the meiotic divisions.

d. *Hieracium flagellare* is a polyploid species, and the reduction divisions show irregularities such as incomplete pairing, lagging, extrusion, polycary, polyspory, and a marked difference in the size of the pollen grains.

2. The species of *Euhieracium* and *Pilosella* exhibit the cytological peculiarities of known hybrids, as well as being polyploid.

3. The species of *Euhieracium* and *Pilosella* develop embryos apomictically.

4. The presence of taxonomic difficulty in the subgenera *Pilosella* and *Euhieracium*, together with the facts of irregular meiosis, polyploidy, and the apomictic development of embryos, is considered as evidence for the hybrid origin of the species of the subgenera here discussed.

5. Hybridism is probably one of the ways by which polyploidy arises, as well as one of the ways by which a multiplication of species is brought about.

This investigation has been carried out under the supervision of Professor E. C. JEFFREY to whom I am greatly indebted for interest and advice.

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STUDY OF THE LIFE HISTORY OF BRASSICA OLERACEA

OSCAR H. PEARSON

(WITH SIXTEEN FIGURES)

Introduction

No detailed morphological study has apparently been made of the flower of *Brassica oleracea* L. This paper reports such a study, and the data apply equally well to all the varieties of the species,—cabbage, cauliflower, brussels sprouts, kohlrabi, kale, broccoli, and sprouting broccoli.

The work on controlled pollination was done at Berkeley, California, during the springs of 1926 and 1930; and the physiological studies on pollen were made at Davis, California, during the winter of 1930–1931. The plants used were cauliflower, broccoli, and cabbage at Berkeley, and sprouting broccoli, grown in the greenhouse, at Davis. The rates of growth and development were secured at Berkeley and Davis, and may only approximate those under other climatic conditions.

Floral development and pollination

The inflorescence is typically racemose, sometimes paniculate. In cauliflower types, however, the inflorescence is abortive. According to LUND and KJAERSKOU (8), this abortion results from a suppression of the terminal growing point which does not at first affect the lower branches. These lower branches grow to about the same level as the terminal growing point, and are in their turn inhibited. In this way a large "curd" is formed, the blanching and fleshy development of the peduncles being caused by their growing in the shade of the wrapper leaves. Later in the season the side and lower branches resume growth, followed by the more nearly terminal ones. The flowers of the cauliflower are sometimes borne in simple racemes; but usually the inflorescence is made more complex by the lack of divergence of several small peduncles for a considerable distance and by the appearance of only a few flowers on shortened racemes.

The smoothed curves in figure 1 have been constructed to represent the typical development of the floral organs of *Brassica oleracea*. The data for this chart were secured by measuring the outer sepal of the same bud daily from as early a period in its development as it could be distinguished. These measurements fixed the curve for the sepals. More than 100 buds were measured in this way. The curves for the buds which developed normally were of the same shape in all cases, although the rate of growth depended somewhat upon the prevailing temperature. A number of buds of various ages were dissected, and the various floral organs measured to secure data for the curves of development of these organs. These curves differ appreciably from those for *Cardamine pratensis* as shown by GUNTHART (5), for they show a sudden expansion of the floral parts, as is seen in figure 1. Anthesis in this case was completed in a single day, the twenty-first day after the flower buds were large enough to measure, as shown on the chart.

The method of origin of the floral parts in Cruciferae was described by EICHLER (4). The outer sepal of the lower whorl opposite the raceme axis is first differentiated. The other organs appear in the usual order of petals, stamens, and carpels. Figure 2 shows a longitudinal section through the tip of a raceme, and the mode of origin of the floral organs.

Two theories have been advanced to describe the ovary in Cruciferae. The generally accepted theory considers that it is made up of two carpels; the other that it is made up of four. SAUNDERS (13) has advanced the idea of polymorphic carpels; the valves being thought to be the sterile carpels, whose margins are adnate to two solid, fertile carpels that are prolonged to form the style and stigma. EAMES and WILSON (3) are in general agreement with this theory, but EAMES (2) considers as improbable the presence of solid fertile carpels.

Anthesis usually starts about four or five o'clock in the afternoon. The rate of growth of the petals and filaments of the stamens at this time increases rapidly; the pressure from these organs finally forces the sepals apart. During the night the growth of these organs continues, and in the morning the petals are nearly full length. They are tightly rolled unless, during the process of opening, the sepals

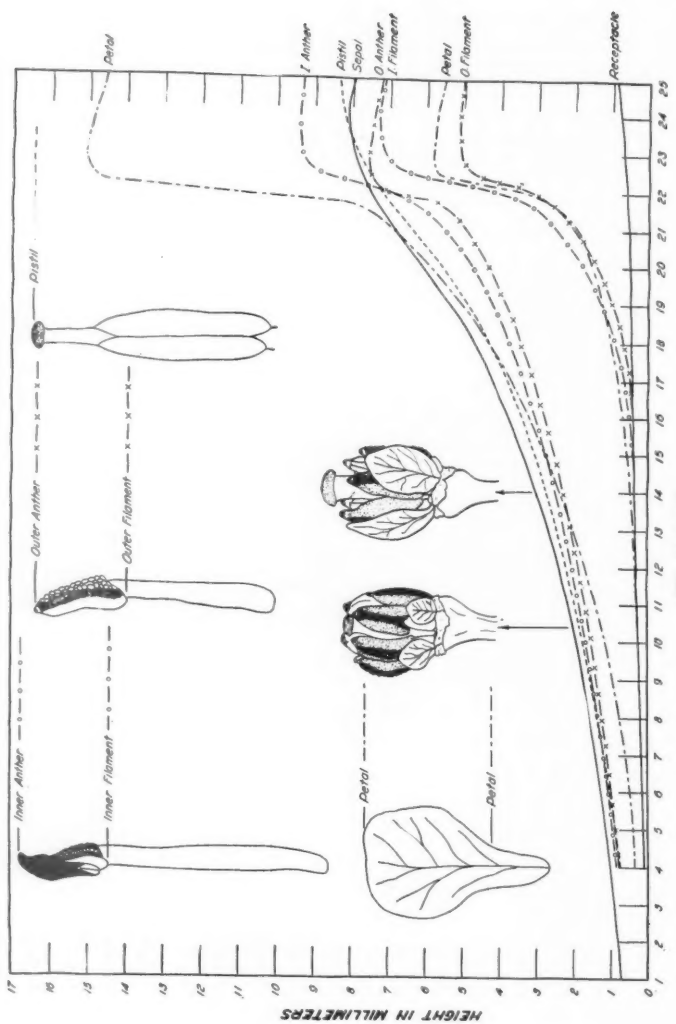


FIG. 1.—Rate of development in flower of *Brassica oleracea*

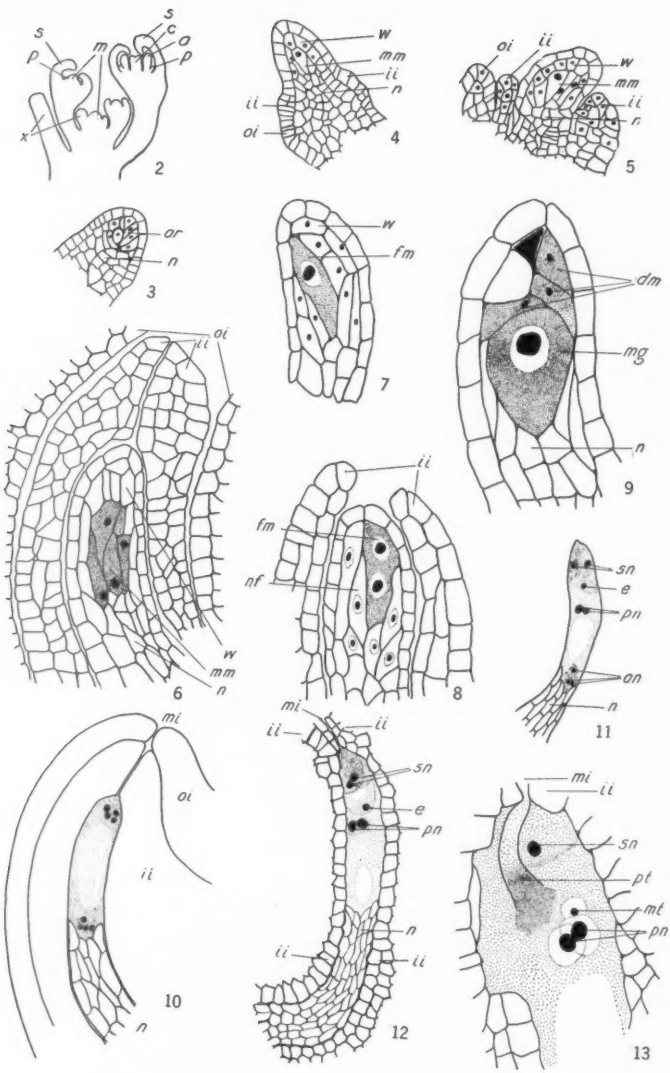
have disarranged them. The flower is usually fully expanded before 10 A.M. Often it opens completely during the afternoon, especially in warm weather. The anthers usually dehisce as soon as the petals begin to reflex.

Brassica oleracea flowers are insect-pollinated. Although ROEMER (**12**) found that kale and kohlrabi pollen is carried by wind, bees are the chief agents of pollen dispersal. Honey-bees, unlike many of the other insects that visit flowers, do not work at temperatures below 60° F., whereas cabbage blooms most profusely at temperatures which for most of the day are somewhat below that. Bumble-bees, although not plentiful in California, are often found on *Brassica* flowers. The solitary bees, closely related to the honey-bee, serve as the chief agents of pollination. Syrphus flies (*Syrphidae*), although occasional visitors to the flowers, are not important pollinating agents. House flies and blow flies are often seen on *Brassica* plants, but they very seldom, except by accident, happen upon either pollen or nectar. HOWARD (**6**) has described the manner in which honey-bees visit flowers of *B. juncea*.

Nectar is secreted in relatively large amounts by the nectaries between the short stamens and the ovary. It amounts to approximately 0.1 cc. per flower each 24 hours, and is secreted until the third day, when the corolla starts to wither. The nectaries outside the two long stamens are functionless.

Microspore

ONTOGENY.—The archesporial cells are distinguishable in buds as young as the largest shown in figure 2. But three or four layers of wall cells are formed, and the entire development is typical of that found in most angiosperms. Nine bivalent chromosomes are present at the first metaphase of the reduction division. This stage occurs in buds about 1.6–1.8 mm. in length. By the time the buds are 2 mm. long the tetrads have been released from their gelatinous matrix. Further development consists of a growth period, followed by a laying down of the exine as a secretion from the tapetal cells, as described by KOSMATH (**7**). The first division of the microspore nucleus occurs two or three days before anthesis, and the division of the generative nucleus the day that the flower opens. At the time of shedding there are three nuclei in the pollen grain.



FIGS. 2–13.—Fig. 2, longitudinal section through tip of a *Brassica* raceme; archesporial cells visible in anther of largest flower bud; ×12.5. Fig. 3, section through tip of ovule showing cluster of archesporial cells directly beneath epidermis; ×200. Fig. 4, section through tip of ovule showing wall cells just formed and nearly as large as mega-

Ovule and megagametophyte

ONTOGENY.—The primordium of the campylotropous ovule can first be distinguished in buds somewhat less than 1 mm. in length. The integuments appear at the base of the nucellus as two concentric rings of actively growing tissue, just before the reduction division in the pollen mother cells; that is, in buds about 1.5–1.6 mm. in length. At the time the microspores are separate in the tetrads, the integuments have reached the tip of the nucellus. Buds about 5 mm. in length show reduction divisions of the megaspore mother cell. Buds 7 mm. in length show the second and third nuclear divisions of the developing megagametophyte, which is completely organized at anthesis.

The development of the megagametophyte in several genera of the Cruciferae has been described by VANDENDRIES (**16**). Megagametophytic development in *Brassica oleracea* is similar to VANDENDRIES' description for *Sisymbrium taraxacifolium*. The tip of the nucellus contains several archesporial cells directly beneath the epidermis (fig. 3); these divide to form the primary wall cells and the megaspore mother cells (figs. 4–6). One of the several megaspore mother

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spore mother cells just beneath; integuments just beginning to appear; ×200. Fig. 5, section through tip of ovule showing wall cells and megaspore mother cells; cell on left apparently beginning to encroach on other mother cells; ×200. Fig. 6, archesporial cells in tip of ovule; wall cells formed but megaspore mother cell not yet encroaching upon other cells; ×440. Fig. 7, section of nucellus showing wall cells and enlarging megaspore mother cell; larger cells beside the large mother cell are also megaspore mother cells, but not functional; ×510. Fig. 8, result of first division of megaspore mother cell nucleus; partitioning cell wall not yet formed; ×510. Fig. 9, one-celled megagametophyte from bud 5.5 mm. in total length; three anterior daughter cells disintegrating; ×775. Fig. 10, end of third division of megagametophyte, with four nuclei at each end of the enlarged cavity; ×290. Fig. 11, megagametophyte just organized with antipodals present; ×220. Fig. 12, fully mature megagametophyte in stage usually found in fully open flowers; antipodal cells entirely disappeared; central core of thin-walled cells is remains of nucellus; cells bordering them and the megagametophyte are epidermal cells of inner integument; ×290. Fig. 13, triple fusion of polar nuclei and sperm, discharged contents of pollen tube completely hiding egg and sperm; ×775. *a*, anther; *an*, antipodal nuclei; *ar*, archesporial cells; *c*, carpel; *dm*, degenerating megaspores; *e*, egg; *fm*, functional megaspore mother cell; *ii*, inner integument; *m*, meristem; *mg*, megagametophyte; *mi*, micropyle; *mm*, megaspore mother cells; *mt*, microgamete; *n*, nucellus; *nf*, non-functional megaspore mother cells; *oi*, outer integument; *p*, petal; *pn*, polar nuclei; *pt*, pollen tube; *s*, sepal; *sn* synergids; *w*, wall cells; *x*, pedicels of buds not shown in section.

cells, usually that near the center of the nucellus, continues to develop, the others remaining passive and being digested or pushed aside by the enlarging megaspore mother cell (fig. 7). The wall cells do not develop further, and are absorbed or pushed aside by the enlarging megaspore. By two successive divisions a linear tetrad is formed (fig. 8); the chalazal megaspore becomes very large; while the three micropylar spores remain small and soon degenerate (fig. 9). These cells are digested and absorbed until finally the one remaining megaspore occupies the entire tip of the nucellus, the parietal cells having been pushed aside or digested. The megaspore nucleus undergoes three successive divisions, and the eight nuclei (fig. 10) become arranged as shown in figure 11. At this time the upper end of the megagametophyte lies in contact with the inner integument, the apical portion of the nucellus having been digested. The antipodals disappear early.

At the center of the ovule there is a distinct core of thin-walled, long, loose cells, light-staining and apparently dead or dying (fig. 12), the remains of the nucellus which extend toward the chalaza from the proximal end of the megagametophyte. These gradually disappear as the embryo sac increases in size after fertilization. VANDENDRIES (**16**) and RIDDLE (**11**) describe a similar tissue. The major portion of the ovule at anthesis is therefore composed of the fleshy inner integument.

Microgametophyte

The surface of the stigma is made up of long papillate cells. At anthesis and while the flower is fully expanded, these cells are moist. Between them is a thick gelatinous substance, as seen in fresh sections stained with aceto-carmine. Just below these papillae are several layers of cells, somewhat elongated at right angles to them. This layer, following the curves of the surface of the stigma, is compacted into a core, similar to the usual stylar canal, directly below the commissural cleft. It continues to the base of the ovary as the central tissue of the partition wall or replum. In this tissue the pollen tubes grow. Full expansion of the stigmatic papillae is not necessary for germination of the pollen. Empty pollen grains with traces of pollen tubes between the unexpanded papillae were found in sectioned flowers pollinated before anthesis.

Age of the ovary has apparently little influence on the rate of pollen-tube growth, for in a bud 4.2 mm. in length, pollen tubes were observed half way down the ovarian cavity 10 hours after pollination, or at about the same place they would have reached had the pistil been mature. In this case, since the ovules in this ovary still contained megaspore mother cells, fertilization could not occur for several days. An ovary killed 151 hours after pollination had, at the time of preserving, mature megagametophytes. It had been pollinated six days before fertilization could occur. Dead pollen tubes, much curled and twisted, were observed on the funiculi, but no fertilization stages were seen.

POLLEN TUBE BEHAVIOR.—The following studies were made in controlled pollinations of emasculated broccoli flowers at Berkeley during May, 1926. The flowers were killed in a weak chromacetic solution and imbedded in paraffin; the sections 8–10 μ thick were then stained in Haidenhain's iron-alum haematoxylin. The ends of the tubes stain heavily and can easily be distinguished.

The pollen, after being deposited on the stigma, remains inactive for a short time. At the end of the second hour, tubes may be found extending to the bases of the papillae. The pollen tube often winds and twists on the surface of the stigma. Finally, growing down the side of one papilla, it enters the tissue of the stylar canal, growing between the cells. Because of the deep-staining nature of the contents of the tip of the pollen tube, nothing can be distinguished as to nuclear behavior.

In pollen tubes grown in sucrose solutions, then killed in absolute acetic alcohol and stained in Delafield's haematoxylin and safranin, according to the procedure described by WEBBER (**17**), the vegetative nucleus is found at the tip of the tube, and the microgametes appear as lighter bodies close together, about 0.05–0.08 mm. from the tip. VANDENDRIES (**16**), however, found that the microgametes precede the tube nucleus. According to VANDENDRIES, the pollen tube emerges from the replum into the ovarian cavity and grows along the surface until it comes in contact with the funiculus of an ovule. It follows this down to the micropyle and passes between the integuments into the megagametophyte. As soon as it enters, the contents are emptied upon the egg, making observation of fertilization difficult. One sperm, however, can be seen with the polar nu-

clei, the three ready to fuse (fig. 13). Fertilization takes place about 16–20 hours after pollination.

PHYSIOLOGY.—BACH (1) studied the germination of pollen of oil mustard (*Brassica rapa oleifera*) in hanging drops of sucrose solution ranging from 2 to 50 per cent concentration. Best results were secured in 15 to 25 per cent solutions. At room temperature the pollen was viable for four days, but did not remain so for eight days. Pollen in a desiccator in a cellar remained viable for several weeks, but it died in a day or two if stored in the open air of a cellar.

The pollen from flowers of sprouting broccoli plants grown in the greenhouse were used in the tests reported here. Agar smears were found to be unsatisfactory because of lack of regulation of humidity. Van Tieghem cells proved to be the most useful. In 10 per cent sucrose solution pollen grains remained inert; in 15 per cent a high percentage of the resulting pollen tubes ruptured; in 20 per cent the growth was apparently normal, long tubes being formed with only a small percentage of broken ones; in 25 per cent the tubes were not so long, although they appeared very similar to those in the 20 per cent solution. In solutions of glucose, no matter what the concentration, the pollen grains exuded a granular-appearing substance (possibly oil droplets); no pollen tubes developed.

Brassica oleracea pollen germinates over a considerable range of temperature. No germination was secured at temperatures below 10° C., but it was excellent at 15° and 20°; at 26° the tubes were noticeably shorter but apparently normal. At 30°, however, the tubes were short and irregular and almost invariably burst. At 35° only a few grains germinated in this way, the rest remaining inert; at 40° C. no germination whatever was secured. In these tests, pollen was taken from an individual freshly opened anther for any series of trials; and several different trials from the same plant and from different plants were run. The results were always the same.

Results of tests of pollen longevity in a constant temperature chamber showed no difference in germinability of pollen from different anthers of the same flower. For any given date the magnitude of germination was about the same. Both high and low temperatures are injurious to *Brassica* pollen in the dry state. At 4° C. the pollen remained viable two days; at 30° and 35°, only one day; at 11°, six

or seven days. At 21°, however, viability sharply decreased after the second day. Maximum longevity of the pollen, therefore, is found in the vicinity of 11° C. The longevity of pollen under greenhouse conditions (60–85° F.) was likewise tested. The flowers that opened each day upon a raceme were tagged, and germination tests were run at one-day intervals up to and including five days. In no case did pollen more than three days old germinate.

To discover whether these findings are also true for pollen applied to stigmas, pollen of various ages was applied to immature stigmas. Immature stigmas were used to avoid technical difficulties, since it has been shown that young stigmas are receptive (PEARSON **9**). The results showed that pollen four days old is a little less likely to grow than fresh pollen, that when five days old it is much weaker, while pollen six days old is impotent. The slightly longer viability in this as compared with the preceding test doubtless resulted from difference in temperature. Unfortunately no tests were conducted to test the influence of humidity upon the viability of pollen. BACH's results (**1**) with turnip seem to indicate that humidity is at least as important as temperature in determining the length of time for which pollen can be stored.

Embryogeny

RIDDLE (**11**), SCHAFFNER (**14**), SOUÈGES (**15**), and VANDENDRIES (**16**) give detailed discussions of the embryo and its ontogeny in Cruciferae. Instead of tracing cell lineage, therefore, an attempt has been made to determine and demonstrate the times at which different stages occur.

ENDOSPERM.—Very shortly after triple fusion takes place, the endosperm nucleus divides. One of the daughter nuclei remains near the zygote while the other migrates toward the antipodal end of the embryo sac. Division follows division in rapid succession. Twenty-three hours after pollination, or about 5 hours after fertilization, three endosperm nuclei were found; 56 hours after pollination, 36 nuclei were found. At this time there were usually eight or ten endosperm nuclei in the micropylar neck of the enlarging embryo sac. These may be closely synchronized in development, since two cases of simultaneous division were found.

Many of the remaining endosperm nuclei are found at the periph-

ery of the sac, slowly digesting the adjoining cells of the inner integument. As already pointed out, the nucellus completely disappears shortly after fertilization. A number of very large, deep-staining endosperm nuclei are found at the chalazal end of the embryo sac, imbedded in a heavily staining granular substance. This substance, obviously a mechanism for transfer of the nutritive material for the embryo, can be seen through the walls of the living ovule as a bright green spot about 12 to 15 days after pollination. VANDENDRIES described this formation as the result of a hypertrophy of the cells of that region. A similar substance is found about the suspensor of the embryo.

DEVELOPMENT OF SEED.—The method employed for the study of seed development consisted of heating ovules of known age in a strong solution of KOH, nearly at the boiling point. The length of time varied from 5 to 20 minutes, depending upon the degree of clearing desired. These ovules were then put upon a slide in a drop of the solution, placed under a microscope, and traced with a camera lucida. The rate of growth of the ovule and embryo as described is that found in the material used, under conditions at Davis.

The central core of the ovule at the time of fertilization is divided into two parts: the portion toward the micropyle is the megagametophyte; that toward the chalaza is the thin-walled, nucellar tissue in the process of being absorbed by the developing gametophyte. The development of the viscid greenish substance at the chalazal end previously mentioned is not shown by the means of dissection employed until the ninth day. In prepared slides, however, the beginning of this so-called hypertrophy can be detected as early as the first few divisions of the endosperm nuclei in that region of the sac. The rate at which the seed increases in size is indicated in figure 16. The outer integument is three or four cells in thickness; the portion of the inner integument which resists the dissolving action of the endosperm nuclei is several cells thicker. These cells are modified in several ways in the formation of the seed coats, as described by PIETERS and CHARLES (10). The final change in color of the seed coats does not occur until about 40 days after pollination.

DEVELOPMENT OF OVARY.—Rate of growth of the ovary or silique closely parallels that of the ovule, but not that of the embryo.

Figure 14 shows the rate of growth of three typical siliques of sprouting broccoli, grown in a greenhouse at Davis in January, 1931, from the time the ovary could be distinguished (fig. 1) until growth ceased. Anthesis occurred on the twenty-eighth day. Ovaries *A* and *C* were pollinated at that time, while ovary *B* was not pollinated until the thirty-second day. The rate at which these siliques grew was followed by means of daily measurements. The number of seeds produced by *A* and *B* was 20 and 26 respectively. Silique *C* produced but 13 seeds, a fact correlated with its lesser length. Maximum growth was reached about 20 days after pollination. The ovules reached their maximum size in about 18 days, but the embryos did not reach maturity until about the thirty-third day after pollination. Ovarian growth is likewise shown to be closely associated with development of the ovule by the parallelism between the curves for ovaries *A* and *B*. No change occurred in *B* until pollination; but once growth of the ovule began, growth of the ovary proceeded normally.

Figure 15 shows a dependence of the ovarian growth upon growth of the ovule. The curves shown on this chart are of typical siliques of broccoli measured daily at Berkeley during 1926. There is evidently a close relation between the number of seeds formed and the length of the silique. The rate of increase in length is the same whether there are 3 or 15 ovules fertilized; but when but a small number are present, growth ceases after a period of time roughly proportional to the number of developing seeds. In these curves, as contrasted with figure 14, the maximum length was reached a day or two later, but the temperature was lower than at Davis.

Elongation of the silique often occurs without the formation of seed, especially under conditions of high temperature or of reduced light. Frequently ovules in such siliques are swollen, the ovules being sometimes apparently undeveloped. Such siliques are usually found late in the blooming season, when the maximum temperatures have been above 35° C. for several days.

The photosynthetic cells of the wall of the silique are concerned in the nutrition of the embryo, apparently furnishing a considerable portion of its food supply, since flowers inclosed in manila paper bags after fertilization developed into siliques containing shrunken seed.

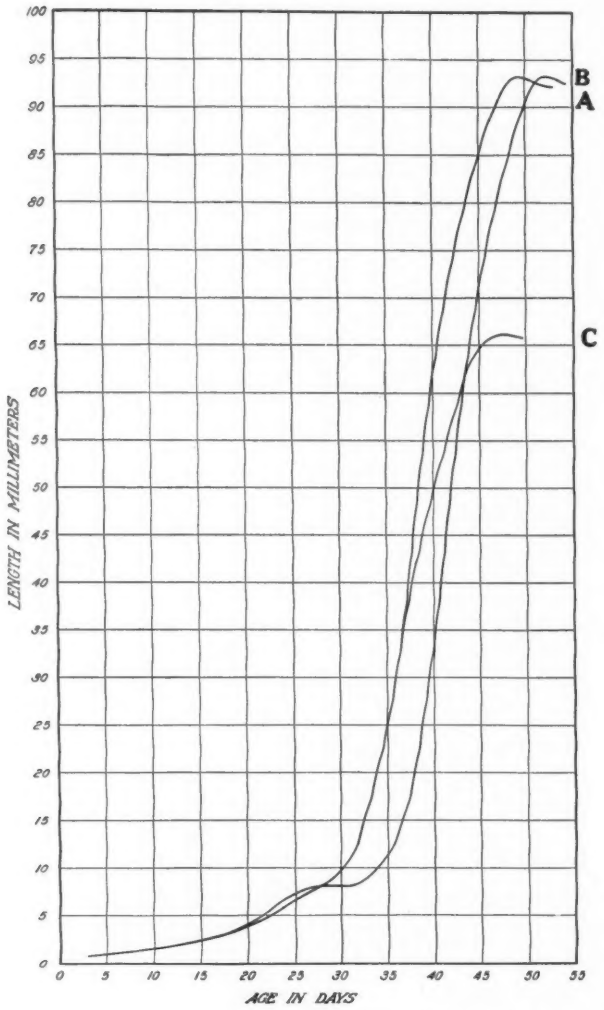

FIG. 14.—Rate of growth of ovary of sprouting broccoli before and after pollination. Ovaries represented by *A* and *C* were pollinated at anthesis; that represented by *B* was not pollinated until four days later.

EMBRYO DEVELOPMENT.—Development of the embryo follows the mode described in other Cruciferae by RIDDLE, SCHAFFNER, SOUÈGES,

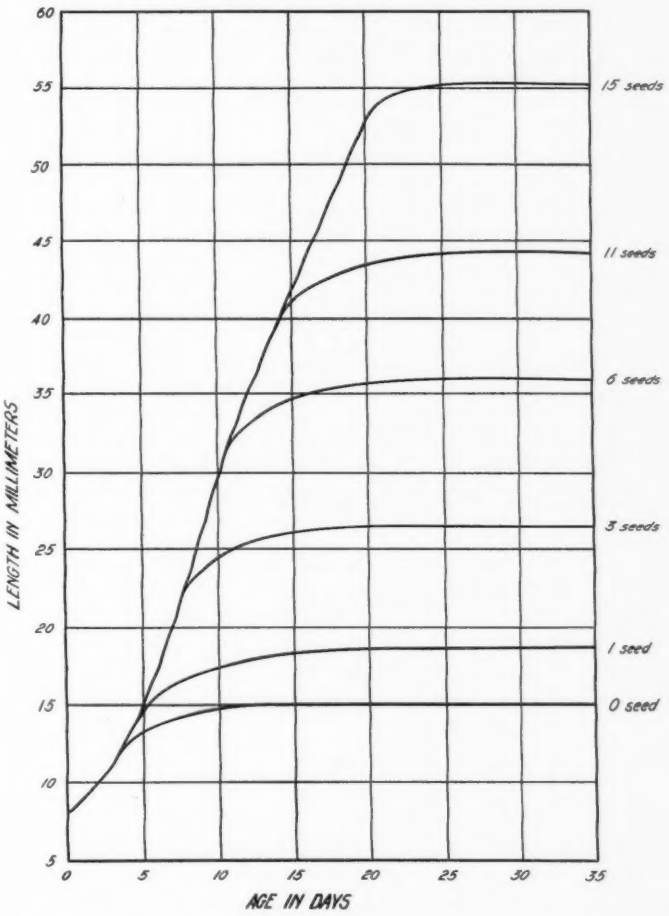

FIG. 15.—Influence of number of seeds upon rate of growth and final length of ovary.

and VANDENDRIES. The zygote does not begin growth until several days after its formation, and until the embryo sac has greatly en-

larged. The first division occurs three or four days after pollination. About the seventh day there are four or five cells in the suspensor and eight cells in the embryo. The cotyledons are differentiated about the thirteenth day. Growth continues in a straight longitudinal direction until about the nineteenth day, when the tip of the embryo strikes the inner wall of the embryo sac. Further growth

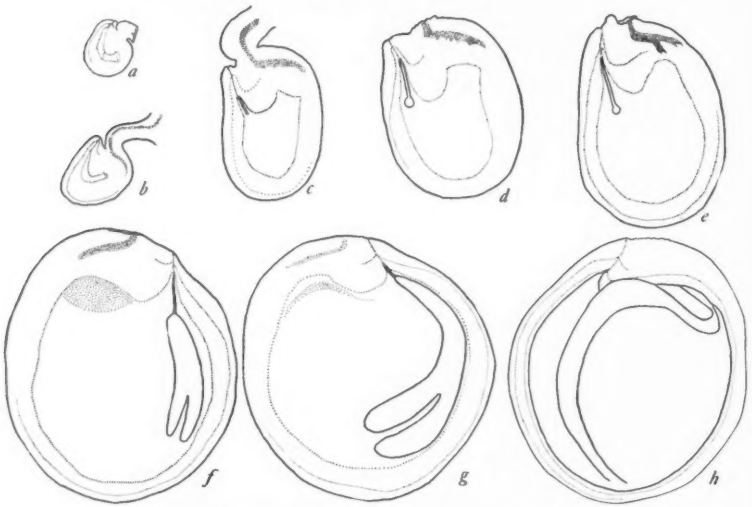

FIG. 16.—Series of drawings, to same scale, of ovules from time of anthesis until embryo is mature; ovule reaches maximum size about 19–20 days after anthesis, if fertilization has taken place. *a*, ovule at anthesis; *b*, ovule after 3 days; *c*, ovule after 7 days; *d*, ovule after 11 days; *e*, ovule after 14 days; *f*, ovule after 19 days; *g*, ovule after 21 days; *h*, ovule after 32 days; ×15.

causes it to become completely curved upon itself; the cotyledons conduplicate. The 7 to 10-celled suspensor in *Brassica oleracea* does not have a swollen basal cell as in *Capsella bursa pastoris* and some other Cruciferae, but resembles that of *Alyssum macrocarpum*. Figure 16 also indicates the rate at which the embryo grows. It reaches maturity 32–35 days after pollination. Ovules produced at Davis were placed in soil 33 days after pollination and 50 per cent of them germinated.

Summary

1. The inflorescence of *Brassica oleracea* is racemose, but in some varieties is modified by lack of divergence to paniculate, as in cauliflower.

2. Anthesis is caused by the rapid growth of petal and filament, which forces apart the sepals, exposing anthers and stigma.

3. Pollination is brought about by insects, chiefly honey-bees and solitary bees in search of pollen, though also gathering nectar.

4. Reduction divisions of the pollen mother cell were found to occur in buds 1.6–1.8 mm. in length. The haploid number of chromosomes is nine. The first division of the microspore nucleus occurs two or three days before anthesis; the second, on the day the flower opens. The vegetative nucleus precedes the microgametes into the tube.

5. Pollen grows readily in 20 per cent sucrose solution at 15°–20° C. The range of temperatures for normal germination is from 10° to 26° C. Pollen is viable about three days at 20° C., but at 30° fails to germinate on the second day, and at 4° fails to germinate on the third day. Good germination was secured after seven days at 11° C. These results were supported by pollination tests in the greenhouse.

6. Several archesporial cells are formed in the ovule. The first division of the megaspore takes place in buds about 5 mm. in length; the second and third in buds about 7 mm. long. Growth of the megagametophyte absorbs or displaces all the distal nucellar tissue, so that the embryo sac lies adjacent to the inner integument. The antipodals disappear soon after the megagametophyte is organized, usually before fertilization.

7. The embryo does not begin development for at least four days after fertilization. The suspensor of a large embryo consists of six to ten cells. Growth of the embryo is slow at first; there are but four cells in the true embryo on the sixth day after pollination. About the thirteenth day the cotyledons appear; about the nineteenth day the embryo becomes curved; and about the thirty-second day the embryo fills the ovule. At Davis, germination was secured from seeds planted and developed within 33 days after pollination.

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CHROMOSOME PAIRING, STRUCTURAL HYBRIDITY, AND FRAGMENTS IN ROSA[1]

EILEEN WHITEHEAD ERLANSON

(WITH TWENTY-THREE FIGURES)

Introduction

The chromosomes of dicotyledons are small, and a true picture of their pairing behavior can be obtained only under optimum conditions of fixation, staining, and microscopy. Evidence in support of the chiasma theory of chromosome pairing (DARLINGTON **6**) has been accumulated from widely separated genera, so that it is now indisputable that chromosome pairing at the first metaphase of meiosis in animals and plants is conditional upon the formation of chiasmata among the chromatids of homologous parts of chromosomes in prophase of meiosis.

Multiple associations of chromosomes may arise from: (1) the presence of more than two homologous whole chromosomes in a nucleus, as in polyploids and trisomic organisms; (2) the presence of more than two homologous chromosomal segments owing to reduplication in diploids and polyploids; and (3) reciprocal translocation (STURTEVANT and DOBZHANSKY **18**), which is also called segmental interchange between non-homologous chromosomes (ERLANSON **12**). References to each type of multiple association have been compiled by DARLINGTON (**8**). Multivalent groups resulting from each of these causes have been found in *Rosa*.

Reciprocal translocation and reduplication

Reciprocal translocation appears to occur rather frequently in diploid roses, and is an important cause of sterility because of the consequent production of non-disjunctional gametes (BEADLE **1**, GAIRDNER and DARLINGTON **13**). The first rose in which it was ob-

[1] Paper from the Department of Botany of the University of Michigan, no. 376, representing work done partially under a National Research Fellowship in the Biological Sciences.

served was the garden hybrid polyantha rose Orleans (species complex *Rosa multiflora* Thunb.), a plant of which had a low percentage of trivalent, quadrivalent, and sexivalent chromosome associations at diakinesis and at first metaphase in the pollen mother cells (ERLANSON 12). A plant of *R. blanda* Ait. (diploid), with similar configurations, was found to be 3x (triploid) for a short chromosomal segment. Quadrivalent groups have since been observed in other individuals of *R. blanda*, as well as in plants belonging to the related diploid species *R. woodsii* Lindl. and *R. pisocarpa* A. Gray (individuals of wild origin). In no rose examined has there been a regular formation of quadrivalent groups in all pollen mother cells at meiosis. This shows, on the chiasma theory of chromosome pairing, that the translocations and reduplications concerned are short segments; they are consequently only rarely able to form chiasmata at diplotene. An analogous situation has recently been reported in *Zea* by BURNHAM (2) and BEADLE (1), and is thought by MÜNTZING (16) to be present in *Galeopsis*.

The diploid roses that show multivalent configurations may have small segments of some chromosomes reduplicated, but my earlier report of whole chromosome reduplication in *R. blanda* was in error (ERLANSON 10). The mistaken interpretation was due to multivalent configurations in conjunction with the presence of univalents. In *Rosa* the unpaired chromosomes may divide at both anaphases of meiosis and give rise to 8-chromosome gametes (n = 7), which, however, seem to be non-functional.

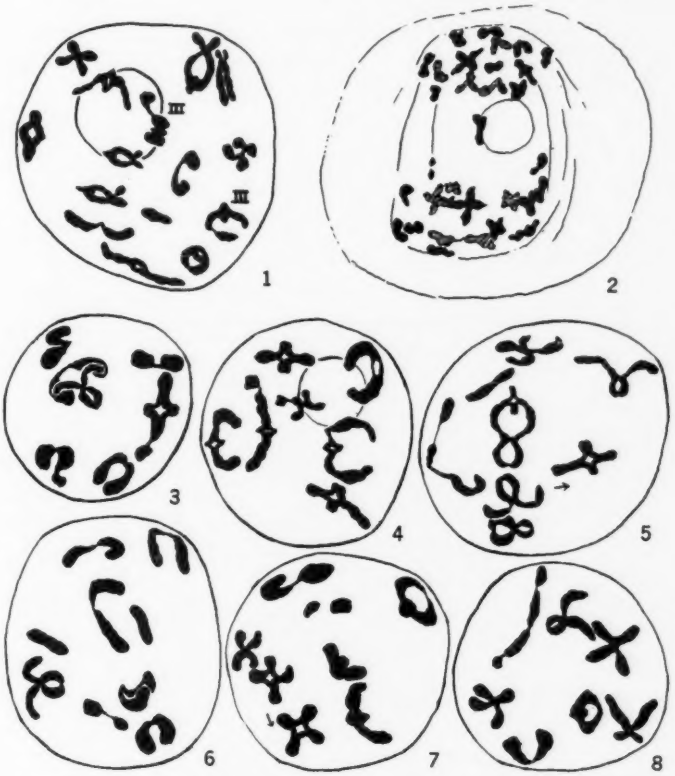
True aneuploidy is very rare in *Rosa*. The two extra chromosomes of the 16-chromosome *R. pyrifera* from Utah (10) are smaller than the others. Their pairing behavior, which is discussed in detail later, shows that they are reduplicated fragments.

An aberrant sterile plant (6424/1)[2], which was raised from seed of the tetraploid *R. arkansana* Porter, obtained from a nursery, was found to have three extra chromosomes which frequently formed trivalent groups at diakinesis (fig. 1). The first anaphase was irregular, and lagging chromosomes sometimes caused a restitution nucleus to be formed at first telophase (fig. 2).

[2] Serial accession number in the Botanical Garden of the University of Michigan.

Chromosomes and fragments in Rosa pyrifera

A plant of *Rosa pyrifera* Rydberg (6610B) from Utah was found to have 16 pairs of chromosomes (**10**). One pair was distinctly small-



FIGS. 1–8.*—Fig. 1, diakinesis in atypical offspring of *R. arkansana* with two trivalents, 12 bivalents, and one univalent. Fig. 2, restitution nucleus at first telophase in atypical *R. arkansana* with 31 chromosomes. Figs. 3–8, diakinesis nuclei in *R. pyrifera* (6610B), 2n = 14+f+f′. Figs. 3, 4, f and f′ paired with different pairs of chromosomes. Fig. 5, $1_{III}+6_{II}+1_{I}$; f and f′ in the trivalent group. Fig. 6, f and f′ paired with each other, also $6_{II}+2_{I}$. Fig. 7, 7_{II}; f and f′ unpaired. Fig. 8, f and f′ paired with different members of the same pair, also 6_{II}.

* Figures drawn with a camera lucida at a magnification of app oximately 6000 and reduced to two-thirds in reproduction; except figs. 9–12 and fig. 22, which were drawn at approximately 4500× and reduced to three-fourths in reproduction.

er than the other seven. This plant is not entirely hardy in Michigan and produces only a few flower buds. Some young anthers fixed in acetic alcohol were cut at 20 μ and stained with Newton's gentian violet method. A sufficient number of whole nuclei at diakinesis were obtained for a detailed study of chromosome pairing at this stage. Although very few nuclei have been observed at earlier or later stages of meiosis, the conditions at diakinesis are similar to those in other roses at the same stage, and are therefore believed to give a reliable idea of the chromosome organization in this plant.

TABLE I

ROSA PYRIFERA (6610/B), 7_{II}+f+f′; ANALYSIS OF CHIASMATA AT LATE DIAKINESIS

No. of divisions observed	No. of potential pairs of chromosomes	Total no. of Xta	No. of Xta terminal	Mean no. of Xta per bivalent	Terminalization coefficient*	Highest no. of Xta in any pair
25	175	221	127	1.27	0.57	3

* Proportion of total chiasmata terminal at any stage (13).

The two small extra chromosomes in the rose 6610B pair not only with each other, but also with two different pairs among the normal set of fourteen. Only homologous parts of chromosomes pair. To interpret chromosome behavior we must assume homologies that are in accordance both with the parts capable of pairing and with the frequencies of pairing. The members of the small extra pair are unequal. They must evidently be considered as fragments rather than as whole chromosomes, since the larger of the two is about half as long as the smallest of the normal chromosomes. The larger is here designated as fragment f′; the other, slightly smaller, is called f.

Chiasma formation was analyzed in 25 whole nuclei at diakinesis in the pollen mother cells (figs. 3–8). As shown in table I, the mean number of chiasmata per bivalent is only 1.27, calculated for the seven normal pairs. Table II gives the frequencies of the same chiasmata (from 1 to 3) in bivalents (in uncut nuclei). As in other roses, there is a failure of chiasma formation in one pair of chromosomes in a small number of nuclei. In table III the individual 6610B of *R. pyrifera* is compared in its chromosome behavior at late diakinesis with the other two roses (12) in which chiasma formation has been

studied. The *R. pyrifera* plant has a slightly lower chiasma frequency than the other two, which is perhaps due to terminalization having proceeded further. The percentage of bivalents lacking chiasmata is similar in all three, yet the *R. pyrifera* has only one chiasma in 70.3 per cent of the bivalents. This is a higher proportion than was shown even at first metaphase by the other two roses. The terminalization coefficient is higher in this rose. The fragments fail to

TABLE II

CHIASMA FREQUENCY FOR 14 WHOLE CHROMOSOMES IN ROSA PYRIFERA AT LATE DIAKINESIS

	No. of chiasmata				Total potential bivalents	Total chiasmata	Mean no. of Xta per bivalent
	0	1	2	3			
No. of bivalents	4	123	46	2	175	221	.....
Percentage of bivalents	2.3	70.3	26.3	1.1	.....	.....	.....
Total no. of chiasmata observed	0	123	92	6	175	221	1.27

TABLE III

COMPARISON OF CHROMOSOME BEHAVIOR IN THREE DIPLOID ROSE INDIVIDUALS AT LATE DIAKINESIS

Name of Rose	Percentage of bivalents failing to pair	Mean no. of Xta per bivalent	Terminalization coefficient	Percentage of bivalents with only one Xma	Highest no. of Xta observed in any bivalent
Rose Orleans	2.6	1.65	0.50	33.12	3
Rosa blanda, 7N29	1.3	1.47	0.47	52.6	3
Rosa pyrifera, B.	2.3	1.27	0.57	70.3	3

pair more frequently than the major chromosomes, as would be predicted on the chiasma theory. In the nuclei studied they have been paired by terminal chiasmata only. As in *Fritillaria imperialis* (DARLINGTON 5), all the chiasmata are near the ends of the fragments, and terminalization is complete at diakinesis in these though not in the longer chromosomes.

To analyze the pairing behavior of fragments, each of them has to be treated as a potential half-bivalent; where they form a chiasma

with each other, or with a chromosome, the individual fragment is credited with a half chiasma (**5, 12**). Table IV shows that the shorter fragment, f, has a higher pairing frequency than its longer mate, f′. The mean number of half chiasmata for f is 1.1, which is a little less than half the mean for each chromosome of the normal set (2.54); while the mean chiasma frequency of f′ is almost a third less than

TABLE IV

PAIRING OF FRAGMENTS IN 25 NUCLEI IN ROSA PYRIFERA (6610B)

No. of 1/2 chiasmata	0	1	2	Total no. of 1/2 chiasmata	Mean no. of 1/2 Xta per fragment	No. of 1/2 Xta terminal	Terminalization coefficient
Fragment f.....	1	20	4	28	1.1	28	1
Fragment f′.....	3	22	0	22	0.88	22	1

TABLE V

TYPES OF PAIRING BETWEEN FRAGMENTS F AND F′ AND CHROMOSOME PAIRS AA AND BB

Pairing arrangement	No. of nuclei
1. AA+f, BB+f′	12
2. AA+f, B+f′	1
3. A+f+A, BB+f′	1
4. A+f+A, f′ free	1
5. AA+f, f′ free	1
6. f+f′	5
7. AA+f+f′	1
8. A+f+f′	1
9. f+AA+f′	1
10. f and f′ free	1
Total nuclei	25

that of the other chromosomes. In 25 nuclei, fragment f′ was free three times, while f was free only once. The reason for this is shown in an analysis of the types of pairing undergone by the fragments (table V). The small fragment, f, pairs with the smallest pair of chromosomes (figs. 3, 4), which I have designated as AA, and f′ pairs with a medium sized pair, BB. The pairing arrangements 1–5, table V, are very similar; the fragments are paired with different whole pairs. Since the homologies of chromosomes are specific to

their parts, the constitution of the parts involved in these pairing arrangements can be represented thus: $AA=\frac{ab}{ab}$, $f=b$, $BB=\frac{cd}{cd}$, $f'=c$. Arrangements 6, 7, 8, and 9, table V, show that f and f′ also pair with each other and with the same pair, AA (figs. 5, 6, 8); therefore one of them consists of two segments. We may assume that this is the larger f′, because it has the lower pairing frequency. The constitutions of the chromosomes and fragments involved are therefore $\frac{ab}{ab}$, b, $\frac{cd}{cd}$, cb.

In prophase, f′ would have difficulty in using both ends at once, and this discontinuity in pairing homology reduces its chiasma frequency (DOBZHANSKY **9**).

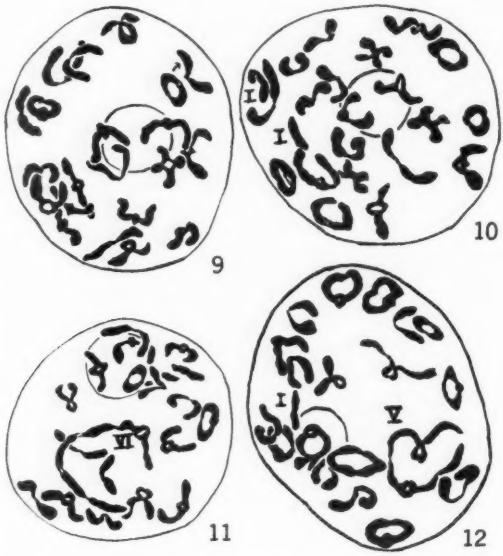
The pairing behavior of these fragments shows that the plant is quadrivalent for a short segment (b above), and trivalent for another small segment (c above). DARLINGTON (NEWTON and DARLINGTON **17**) has pointed out that short fragments are better testers of homologies because they are not carried away by long continuities of homology. Because f′ consists of two segments, cb, both fragmentation and translocation must have taken place. The configuration $\frac{ab}{ab}+b+bc+\frac{cd}{cd}$ has not been observed; it would be expected to occur infrequently if the translocation c be rather short. Changes of homology arrest terminalization (**12**), and it is possible that when they are present the process of terminalization causes a strain which might cause fragmentation; but in *Rosa* and *Oenothera* (DARLINGTON **7**) such arrest does not lead to breakage of chromatids. There is no evidence to indicate that translocations tend to occur at the same locus in particular chromosomes (**2**).

This is the only instance of fragments that has been found in *Rosa*. DARLINGTON (**3**, **4**) predicted that effective fragmentation would be rare in the great plant genera *Rubus*, *Ribes*, *Rosa*, *Prunus*, *Avena*, and *Triticum*. Fragmentation is not absent, but when it occurs it is usually eliminated in the course of sexual reproduction. It may become fixed by apomixis, however, as in *Potentilla* (MÜNTZING **15**). This plant of *R. pyrifera* has 40 per cent of the pollen grains either dwarfed or shriveled.

Rosa pyrifera belongs to the species complex of *R. woodsii*, from which it differs chiefly in having pyriform hips and pubescent foliage.

Pairing in higher polyploids

Multiple chromosome association occurs in all the balanced tetraploid, hexaploid, and octoploid roses I have examined. Tetraploids frequently show one or two quadrivalent groups in 50 per cent or more



FIGS. 9–12.—Diakinesis in F_1 of *R. engelmanni*×*nutkana*, 2n=42; nuclei with 21_{II}, $20_{II}+2_I$, $1_{VI}+18_{II}$, and $1_V+18_{II}+1_I$ respectively.

of the nuclei at diakinesis and first metaphase (**10, 12**). Non-disjunction is frequently associated with rings and chains of chromosomes, and this is probably one of the causes of the relatively high sterility found in tetraploid species of *Rosa* (**11**), as in autopolyploids generally (**8**). Multivalent chromosome groups are less frequent in polyploids higher than tetraploid. This may be due to a difficulty in obtaining maximum association for all pairing possibilities among sexivalent homologous segments in prophase, on a basis of random chi-

asma formation in these short chromosomes (**13**). In hexaploids there are 21 pairs in a majority of nuclei at diakinesis (fig. 9); occasionally $20_{II}+2_{I}$ appear (fig. 10). More than one chain of five or six has not been observed in one nucleus (figs. 11, 12), although as many as four quadrivalent groups have been found (ERLANSON **10**, fig. 63).

Figures 9–12 are diakinesis configurations in an F_1 individual from *R. engelmanni* (6x) × *nutkana* (6x). The male parent was obtained from the Priest River region of northern Idaho, and the female parent from the Medicine Bow Mountains of Wyoming. The offspring are hexaploid, have only 10 per cent of the pollen empty, set good fruit, and would be classified as *R. engelmanni* by a taxonomist.

The multivalent configurations considered in this section are presumably due to the presence of more than two homologs in the nuclei of the polyploids. If reciprocal translocations are present and are of sufficient dimensions to form chiasmata, then associations of more than four chromosomes appear in tetraploids, as in *R. relicta* (**12**). Associations of more than six chromosomes have not been found in hexaploids.

Chromosome pairing in unbalanced polyploids

TRIPLOIDS.—The first wild triploid roses that I reported (**10**) were spontaneous hybrids between diploid and tetraploid species. They usually had seven paired and seven univalent chromosomes at diakinesis. A triploid has since been found in a culture of diploid *R. blanda*, from Michigan. This plant probably originated in the fusion of a diploid with a normal haploid gamete of the same species. It had a high percentage of trivalent groups at diakinesis and first metaphase of meiosis; different nuclei showed from three to seven trivalent groups. This variation is characteristic of non-hybrid triploids, and is expected on the chiasma theory of pairing (**8**). Figure 13 shows a first metaphase with five trivalents and two univalents. Figure 14 shows the end of first anaphase with six chromosomes at one pole, eleven at the other; one pair lagging and two univalents dividing at the equator.

In a culture of diploid *R. macounii* Greene (species complex *R. woodsii*) from Reno, Nevada (no. 12205/15), which was grown from seed of a single individual and cultivated at the California Institute

of Technology, one plant among 21 was found to have 90 per cent of the pollen shriveled. Cytological examination showed that it was a triploid with a high proportion of trivalents at diakinesis. Among ten whole nuclei, three had seven trivalents (fig. 15), five had $6_{III}+1_{II}+1_{I}$, and two had $5_{III}+2_{II}+2_{I}$. This plant also probably arose from an unreduced gamete.

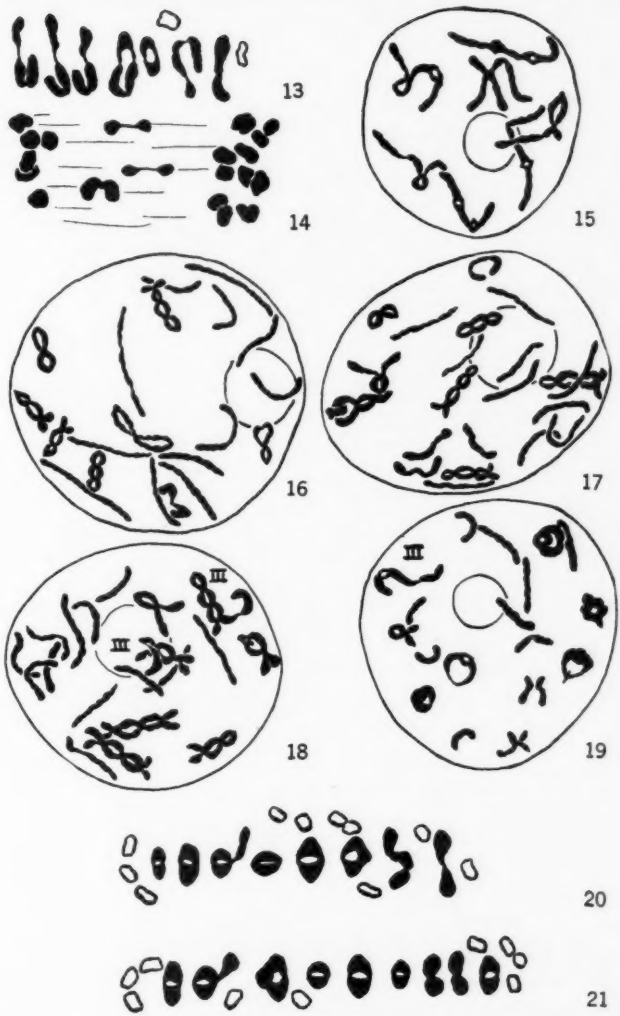
A seedling of *R. pisocarpa* Gray (12259) with sterile pollen and ovules was also triploid with 21 somatic chromosomes, and had a high percentage of trivalents at diakinesis. The parent plant grew near Jackson, Oregon, and was laden with fruit; it was presumably a normal diploid.

TABLE VI

TYPES OF PAIRING IN 20 NUCLEI OF ROSA VILLOSA

PAIRING ARRANGEMENTS	NUCLEI AT EARLY DIAKINESIS	NUCLEI AT LATE DIAKINESIS	NUCLEI AT METAPHASE
$7_{II}+14_{I}$	1	3	1
$1_{III}+7_{II}+11_{I}$	1	3	1
$1_{III}+6_{II}+13_{I}$	.....	1	.....
$2_{III}+5_{II}+12_{I}$	1	.....	.....
$2_{III}+6_{II}+10_{I}$	.....	.....	1
$8_{II}+12_{I}$	.....	6	.....
$9_{II}+10_{I}$	.....	1	.....

A TETRAPLOID IN SECTION CANINAE.—Chromosome pairing was analyzed in a few whole nuclei of the unbalanced tetraploid *R. villosa* L. This rose belongs to the section Caninae, all the members of which have been reported by TÄCKHOLM, HURST, HARRISON, and BLACKBURN to be unique in never having more than seven bivalents at diakinesis and first metaphase. On the chiasma theory of chromosome pairing, one would expect occasional variations in this arrangement owing to the presence of more than two homologous chromosomes. Table VI shows the types of chromosome pairing actually found in 20 nuclei at early and late diakinesis and at first metaphase. The chromosomes are small and it is difficult to analyze pairing conditions, especially at early diakinesis and metaphase. These few figures, however, show clearly that $7_{II}+14_{I}$ appear only in a minority of the nuclei. In these 20 nuclei, five had $7_{II}+14_{I}$ (fig. 17), six had $8_{II}+12_{I}$ (fig. 16), and five had $1_{III}+7_{II}+11_{I}$ (fig. 19). One cell had $9_{II}+10_{I}$. Trivalents were found in eight nuclei.



FIGS. 13–21.—Fig. 13, *R. blanda* (7N44), 2n = 21, first metaphase with $5_{III}+2_{II}+2_{I}$. Fig. 14, *R. blanda* (7N44), 2n = 21, first anaphase, two univalents dividing at equator, one pair lagging. Fig. 15, *R. macounii*, 2n = 21, diakinesis with 7_{III}. Figs. 16–18, nuclei of *R. villosa*, 2n = 28, in early diakinesis with $8_{II}+12_{I}$, $7_{II}+14_{I}$, and $2_{III}+5_{II}+12_{I}$ respectively. Fig. 19, *R. villosa*, late diakinesis with $1_{III}+7_{II}+11_{I}$. Fig. 20, metaphase complement in *R. villosa* with $2_{III}+6_{II}+10_{I}$. Fig. 21, metaphase complement in *R. rubrifolia*, 2n = 28, with $1_{III}+8_{II}+9_{I}$.

In the early diakinesis nuclei shown in figures 16, 17, and 18 there are clearly 14 chromosomes with a high chiasma frequency, and 14 with a very low chiasma frequency which may be associated with each other or with the regular pairs by terminal chiasmata only. The contrast between the chiasma frequencies of these two sets of 14 chromosomes is shown graphically in figure 23. In three nuclei at early diakinesis there was a total of 67 chiasmata among the seven normal pairs, and only 22 of them were terminal (table VII). The

TABLE VII

ANALYSIS OF CHIASMATA IN ROSA VILLOSA

STAGE	NO. OF NUCLEI	NO. OF PAIRS	TOTAL NO. OF XTA	MEAN NO. OF XTA PER BIVALENT	NO. OF TERMINAL XTA	TERMINALIZATION COEFFICIENT	HIGHEST NO. OF XTA OBSERVED IN ANY PAIR
Early diakinesis..	3	21	67	3.2	22	0.33	4
Diakinesis							
slide 1.....	6	42	110	2.6	46	0.42	4
slide 2.....	8	56	111	1.98	58	0.52	3
Metaphase.....	3	21	37	1.76	33	0.89	2

mean chiasma frequency of 3.2 per bivalent is the highest yet found at this stage of prophase in a rose. In eight cells in the same loculus at late diakinesis, the seven regularly pairing bivalents were again distinguished (fig. 19), and the mean number of chiasmata per bivalent was found to be 1.98. In table VII it is shown that the process of terminalization of chiasmata in *R. villosa* goes on as in other roses (12). As meiosis proceeds, the mean number of chiasmata per bivalent decreases but the proportion of total chiasmata that are terminal increases. At first metaphase terminalization is practically complete (fig. 20), and the terminalization coefficient is 0.89 (table VII). This may be due to a genetic property or to the absence of structural hybridity, as between the normally pairing chromosomes among the modern roses of the Caninae.

A metaphase plate with $1_{III}+8_{II}+9_{I}$ in *R. rubrifolia* Vill., another member of the Caninae with $2n=28$, is shown in figure 21.

Figures 16, 17, and 18 show that the univalent chromosomes at diakinesis are noticeably less condensed than their paired fellows. It has always been difficult to understand why hybridity should pre-

vent the pairing of one-half of the complement while not affecting the other. On the precocity theory of meiosis, and the theory of the origin of sex chromosomes which follows from it (DARLINGTON **6, 8**),

FIG. 22.—Somatic metaphase from root tip of *Rosa* sp., 2n = 35, section Caninae

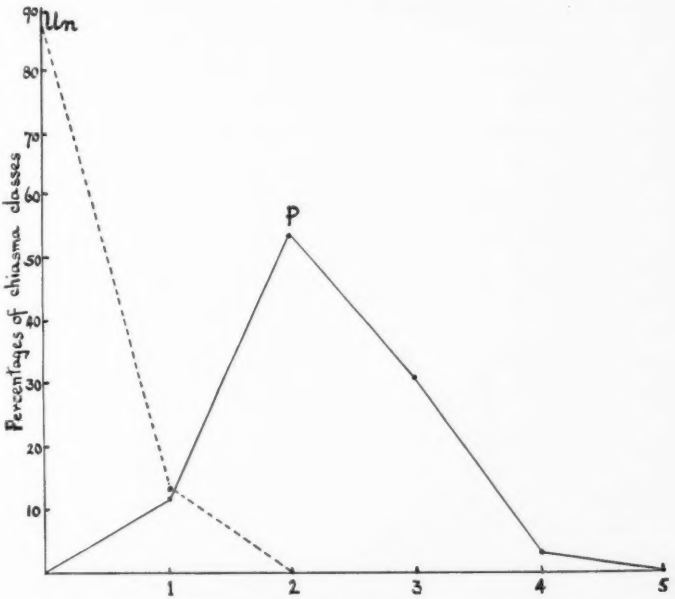


FIG. 23.—Percentage frequency polygons of numbers of chiasmata in bivalents (or their equivalents in unpaired chromosomes and multivalents) in the two sets of chromosomes of *R. villosa; p*, paired set, *un*, unpaired set. Data from 14 nuclei at late diakinesis.

this is intelligible; for it may be supposed that the 14 unpaired chromosomes have a genetic property of differential precocity producing the same effect as that in the sex chromosomes in animals.

Thus the possibilities of genetically caused abnormal zygotene pairing are as follows:

1. *Incomplete precocity* of the prophase (for example, *Matthiola*). The prophase is intermediate in precocity between a normal mitotic and normal meiotic condition; this is expressed in long chromosomes at first metaphase, that is, reduced condensation.

2. *Differential precocity*, the two known types of which are: (a) In *sex chromosomes* there is a differential precocity of the chromosomes themselves. The unpaired sex chromosome is itself precocious, as is the prophase, and so restores normal mitotic conditions for itself. This is expressed in precocious condensation and precocious splitting as in ordinary mitosis (DARLINGTON **6**). (b) In the *unpaired chromosomes* of the Caninae the prophase contraction begins later than in their paired fellows. They presumably have divided before they condense and therefore cannot pair; a condition that was suspected by HUSKINS (**14**) to be a cause of asynapsis in dwarf oats and in sorghum.

Somatic chromosomes of Caninae

A somatic metaphase plate from a root tip of a member of the Caninae is shown in figure 22. This plant, still a seedling, was raised from seed collected in Persia by C. D. DARLINGTON. It is a pentaploid with 35 somatic chromosomes. These show both primary and secondary constrictions, as in diploid roses, and resemble the chromosomes of *Oenothera* (DARLINGTON **7**, fig. 22).

Summary

1. Multiple pairing of chromosomes due to reduplication and reciprocal translocation of segments has been found in diploid roses belonging to the species complexes *Rosa multiflora*, *R. blanda*, *R. woodsii*, and *R. pisocarpa*. Gametes with eight chromosomes are sometimes produced, but no diploid has been found possessing a reduplicated whole chromosome.

2. A plant of *R. pyrifera* Rydb. (group of *R. woodsii*), previously reported to have $2n = 16$, has a small unequal pair which are really reduplicated homologous fragments of approximately half a chromosome. They pair with each other and with two different pairs.

3. Both fragments fail to pair in a proportion of cases. The larger, f′, consists of two segments, homologous with two different pairs. This reduces its pairing frequency (in agreement with the chiasma theory of chromosome pairing) to less than that of the shorter fragment, f. The plant is 4x for one short segment and 3x for another. This is the only example of fragments yet found in *Rosa*.

4. Multivalent associations of chromosomes are frequent among tetraploid roses, but are relatively rare in hexaploids and octoploids.

5. Triploids with a high proportion of trivalents at diakinesis and first metaphase have been found in diploid cultures of *R. blanda*, *R. macounii* (group of *R. woodsii*), and *R. pisocarpa*. These probably originated from unreduced gametes and arose without hybridization.

6. The unbalanced tetraploid *R. villosa* (section Caninae) shows a high chiasma frequency among 14 chromosomes and a very low frequency among the other 14, which usually occur as univalents. In 75 per cent of the nuclei examined, from one to four of the latter were paired terminally with each other or with one of the regular pairs. Trivalents were found in eight nuclei out of 20. Failure of pairing of the chromosomes is probably due to differential precocity in prophase development, not to hybridization.

7. The chiasma frequency of the fourteen regularly pairing chromosomes in *R. villosa* is 3.2 chiasmata per bivalent at early diakinesis, and terminalization is almost complete at first metaphase.

8. The types of structural change and the different kinds of polyploids found in *Rosa* are described. The exceptional types of pairing found are in conformity with the chiasma theory of pairing and are in three instances such as would be predicted on that theory.

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DIVISION OF THE GENERATIVE NUCLEUS IN THE POLLEN TUBE OF LILIUM

JOSEPH O'MARA

(WITH PLATE IX AND SIX FIGURES)

Most cytological investigations of the higher plants during recent years have been concerned with mitosis in sporophytic generations, particularly behavior at meiosis. The first important investigation dealing with the haploid generation was that of STRASBURGER (3). Later NAWASHIN (2) investigated *Lilium martagon*. WELSFORD (5) studied the formation of sperm nuclei in *L. auratum* and *L. martagon*. TRANSKOWSKY, with material artificially grown on agar-agar, determined the gametophytic development of *Convallaria majalis*, *Galanthus nivalis*, and *Hemerocallis flava*.

MATERIALS AND METHODS.—The figures upon which the present study is based were obtained from pollen tubes of *Lilium regale*. Flowers were pollinated in the morning and worked upon at different times during the following day. Since there is considerable variation in the rate of development of the tubes, all stages were usually found in the preparations of a single day. The material was prepared according to the following technique.

The styles and stigmas were removed from the flowers by breaking the style at the top of the ovary. A longitudinal slit was made through the style, which was then opened up, exposing the pollen tubes. These were removed with a pair of fine forceps and laid on a dry clean slide. The slide was then immersed in the fixative and left for two or more hours. The most satisfactory preparations were obtained when Darlington's modification of the Flemming solution was employed as the fixative. A much sharper stain was obtained by bleaching in a 10 per cent solution of hydrogen peroxide in 80 per cent alcohol than was obtained by the usual bleaching in an aqueous solution of hydrogen peroxide. After the material had been sufficiently bleached, it was stained with crystal-violet-iodine.

All drawings, except figure 4, were made using the 100× fluorite objective, 15× ocular, and a Zeiss camera lucida.

POLLEN-TUBE DEVELOPMENT IN LILIUM

Upon germination of the spore, the characteristic tube appears from the fissure of the ruptured exine and grows rapidly down the hollow interior of the style. The tip of the tube is characterized by a slight swelling and hyaline area. As the tube elongates, the vegetative nucleus usually enters it and passes down, followed at a variable distance by the generative cell, which can be distinguished by its darker cytoplasm and nucleus. Between the two cells can be observed a sharp line of demarcation which is apparently due to the different fixation reactions of the generative and vegetative cells. The generative cell is long, with either pointed or blunt ends, and nearly fills the cross-section of the tube. The generative nucleus is ellipsoid in the beginning and is in an early prophase stage even when it first enters the pollen tube. The vegetative nucleus has no constant shape, however, and seems to change its form as various forces act upon it, that is, forces such as pollen tube contour, cytoplasmic streaming, and generative cell proximity. The vegetative nucleus is variable in behavior as well as in form. It may pass through the tube before, after, or may even accompany the generative cell (TRANSKOWSKY 4). Usually it precedes the gametic cell and rarely follows. When it accompanies this cell, it is constricted up against the pollen tube, so that its length may exceed the total length of the generative cell (fig. 7). The vegetative nucleus has weak staining properties, and shows no structure but that of a fine reticulum of anastomosed chromatin threads. The generative nucleus shows strong basic staining properties, however, and can be sharply stained at the earliest stages. Figure 7 illustrates the relative staining properties of these nuclei. The chromosomes of the generative cell are double from the beginning and are extremely long and convoluted, twisting and winding about through the interior of the nucleus very intricately (fig. 1). As the cell passes down the tube it increases greatly in length, and its nucleus also increases in a like manner, so that often a nucleus will have a diameter of 4 μ and a length of 90 μ. The nucleus may increase in size to such a degree that it occupies the major portion of the cell. The chromatin threads become more and more loosely arranged and thicker in diameter. This nucleus resembles very remotely that of the characteristic somatic cells, and its chromo-

somes show none of the structural regularity which is so common in root tip mitoses. The threads at prophase are not solid double rods of chromatin which are bent through the interior and around the periphery of the nucleus, but represent thin, long, granular threads which are loosely wound and twisted through the nuclear interior (fig. 1). Although the fixing and staining seemed to be rather sharp, no granular structure, such as has been illustrated by many investigators, could be observed. The successive stages of prophase are passed through, and finally the nuclear membrane disappears and the chromosomes lie in the generative cell cytoplasm (figs. 2–4). At this stage the behavior differs considerably from that usually met with in connection with dividing nuclei.

The chromosomes of the generative nucleus, instead of forming an equatorial plate immediately upon the disappearance of the nuclear wall, remain in their same relative positions in the cytoplasm of the generative cell. The threads at this stage vary greatly in length in different cells, or even in chromosomes of a single cell.

Twists among the threads of an individual chromosome are frequent. Every chromosome, because of its length and involved association in the long nucleus, has usually one twist, and some chromosomes have been observed with as many as six such associations (fig. 8).

The split chromosomes are very long and are often much intertwined, even at this late stage (figs. 2–4, 9, 10, 12, 13), but the twelve pairs can usually be counted easily. This early metaphase stage seems to persist for a comparatively long time, since figures of this stage are common in all preparations. The threads usually lie longitudinally in the tube, and exhibit many interesting features. The paired threads are not straight rods but have more the appearance of wavy lines when viewed from a position where they seem to be single (figs. 2, 12). The pair of threads, not the half-chromosomes individually, follow this wavy course. If the two threads lie parallel and in the same optical plane, they often seem to be paired rows of chromomeres. This appearance is due to the fact that only the crests of the wavy chromosomes are in sharp focus, and the space between the crests forms a hazy bridge. Thus the chromosomes seem to be paired rows of chromomeres. NAWASHIN and TRANSKOWSKY, how-

FIGS. 1–6.—Fig. 1, metaphase; no equatorial plate nor trace of spindle fibers; fig. 2, metaphase; 12 chromosomes in single line in generative cell; fig. 3, metaphase; outline of pollen tube indicated; twists in sister-threads common; fig. 4, telophase; chromosome groups drawn close together in order to have whole figure on plate; ten Vs and two rods present under normal conditions; fig. 5, small portion of sperm nucleus showing solid convoluted threads which appear after reorganization of telophase clump; fig. 6, long, thin, and twisted double threads observable at earliest stages of prophase; chromosome interchange could very easily take place at this stage also. ×1550.

ever, have illustrated chromomeres in *Lilium* and *Galanthus*. The chromosomes seem to vary greatly in length at this stage.

One generative cell had chromosomes 18 μ in length, and another had chromosomes of 38 μ. This seems to differ from the somatic and meiotic behavior, wherein the chromosomes seem to have a constant length. The somatic chromosomes of this species are approximately 16 μ in length, and the meiotic, approximately 8 μ at metaphase. The positions taken by the chromosomes are also variable; some cells contain compact masses of chromosomes which are impossible to differentiate. In rare cases the chromosomes are strung out linearly, so that a line of twelve individual chromosomes passes from one end of the generative cell to tne other (fig. 3). As the time for division approaches, however, the chromosomes seem to form a more compact arrangement and to attempt the formation of an equatorial plate. Where the tube is narrow, however, there are obviously more chromosomes than can be accommodated by its small diameter. In these instances the chromosomes form a compact cylindrical mass of chromatin whose individual constituents cannot be identified (fig. 14). Pairs of chromosomes which could not possibly lie on a plate are frequently observed, and it seems to be true that no normal equatorial plate is present. Some tubes, because they are wider at the generative cell, allow for a looser metaphase association, and in these tubes the relationships are much clearer. In these instances the chromosomes orient themselves so that their constriction points are in an approximate plane (fig. 9), although figures where these constrictions can be seen to lie in exactly one plane have never been found. The chromosomes show definite constrictions, but not the slightest evidence of spindle fibers is present. STRASBURGER and HERRIG (1), however, have reported that an achromatic figure is formed in other species of *Lilium*, and that definite spindle fibers of short duration are formed. NAWASHIN and TRANSKOWSKY, working with *Lilium martagon*, *Galanthus nivalis*, and *Convallaria majalis*, found no spindle fibers nor achromatic figures. From his own observations on *Hemerocallis flava*, TRANSKOWSKY has concluded that the absence of achromatic figures and spindle fibers is due to the "Raumverhältnisse" of the generative cell and pollen tube. After the actual division, the chromosomes should pass through the tubes with the constrictions foremost. That they do not do so is interesting.

The meiotic chromosomes of *Lilium regale* have ten approximately median and two terminal or subterminal constrictions. The spindle fibers are usually attached to the chromosomes at these points. In this material, however, no spindle fibers could be observed at any stage of division. Not only were these attachment fibers apparently lacking but no evidence from the chromosomes themselves indicated that they were being "pulled" from the middle of the division figure (figs. 6, 14). No stretched portion of the chromatin was observed at or near the constriction, nor did the figures indicate that any force whatever was acting upon the chromatids. The individual chromosome configurations are very variable and peculiar when compared with somatic chromosomes. Threads are also single at this stage, and show no sign of the double structure which has been observed in somatic chromosomes at anaphase (fig. 6).

After the chromosomes reach opposite ends of the generative cell, they form the characteristic clump which is so common at the telophase of somatic divisions. The generative cell seems to elongate somewhat during this division, and now has a long constriction in the area between the two chromosome groups. The clumps begin to loosen slowly and to assume the more usual form of a resting nucleus. The threads become more and more loosely associated and the nucleus increases in size. The exact behavior of the generative cell at this point is difficult to determine. Often the constriction in its center seems to separate the generative cell into two sperm cells. In a great many cases, even when the sperm nuclei were very far from each other, a thin strand of generative cell plasm could be seen connecting them. Regardless of which process the generative cell undergoes, two naked sperm cells are soon found in the cytoplasm of the vegetative cell. As the chromosomes which clump at telophase begin to loosen and form a new nucleus, the cytoplasm of the generative or sperm cell begins to merge slowly with that of the vegetative cell.

No figures were found which gave any indication as to exactly how this dissolution takes place. That it does so is evidenced by the facts that the outline of the generative cell becomes more and more difficult to follow and that naked sperm nuclei are found in the tubes and at fertilization. The cytoplasm of the vegetative cell appears vacuolate at this stage, especially in the region toward the spore. The two

sperm nuclei lie in the cytoplasm of either the generative, sperm, or vegetative cells, and retain a remarkably constant form considering the forces which may act upon them. They are usually ellipsoid and from three to five times as long as broad. These nuclei have peculiar features also.

No characteristic somatic nuclei are formed as a result of the division of the generative nucleus. Instead of emerging from the telophase clump as a reticulum of finely anastomosed threads, these threads retain the more general features of their structure as chromosomes. The sperm nuclei can be seen to consist of solid, single, individual threads which are more like prophase than telophase chromosomes (figs. 5, 10). The sperm in this species is not to be regarded as a typical somatic nucleus, therefore, but more as a nucleus which resembles closely the interphase stage of meiosis. As the nucleus passes down the tube, it becomes more and more reticulate but never reaches a condition of such complete reticulation as is found in somatic nuclei. An examination of fertilization figures showed that, even at the late stages just preceding fertilization, no typical anastomosed sperm nucleus is ever formed. NAWASHIN has demonstrated that the sperm nuclei of *Lilium martagon* do not pass through a resting stage.

Discussion

GENERATIVE CELL DIVISION

One of the most interesting factors in the division of the gametic cell of *Lilium regale* is the apparent absence of an equatorial plate. In no figure examined was the formation of such a structure observed. Although the variations in chromosome behavior seem to be relatively great, a regular distribution of twelve chromosomes to each pole was observed. In all telophase figures examined, twelve chromosomes could be easily counted at each pole. The haploid number of all plants examined was twelve. These two facts, the regularity of division and the maintenance of a constant specific number, establish definitely the regularity of the generative cell division. This division is perfectly normal, therefore, and is accomplished without the formation of an equatorial plate. In view of these few facts, an equatorial plate would seem to be an unnecessary factor in

the process of mitosis. There are, however, certain other factors involved. Certain configurations in the cells suggest that an attempt was made at the formation of such a plate (fig. 9); but at the same time it was also definitely established that in no case was a recognizable plate formed. However, a diagonally oriented plate would present a condition which would allow for plate formation. No such arrangement was found, and in many cells such a structure was apparently impossible (figs. 4, 13). The remaining explanatory factor seems to be that a plate actually is formed, but the movements of the generative cell, in passing down the tube, disrupt it and scatter the chromosomes. That such a situation is possible but very improbable is indicated by all figures examined. Occasionally the plate could be observed, but at no stage was there a chromosome arrangement explainable by the assumption of such a structure. In view of these facts, the conclusion apparently must be drawn that an equatorial plate is not a necessary and indispensable structure or condition in the process of nuclear division.

Not only is the plate lacking, but at no stage in the complete division could the presence of spindle fibers be demonstrated. Numerous figures were examined for evidence of these threads, and in no case could they be detected. In all the metaphase and telophase figures observed, the chromosomes gave no indication that they were being separated and drawn to the poles by any connecting thread. The chromosomes exhibit, in certain preparations, fiber constriction points both at metaphase and telophase. Since in ordinary cells the spindle fibers seem to be attached to the chromosomes at the constricted points, and these points are usually foremost in passing to the poles, the actual separating force, regardless of its nature, may be assumed to be active (or most active) at these points. That this species has ten median and two terminal or subterminal constrictions has already been established. If the division were a normal one, the chromosomes would orient themselves so that their constriction points would lie on the equatorial plate and would then separate first at these points. This behavior would result in a definite and constant telophase arrangement. The chromosomes which possessed terminal or subterminal attachment points would pass to the poles as straight rods, and those which had median attachment points

would separate into Vs. In this particular species ten Vs and two rods should be present in every telophase set of chromosomes. Furthermore, in normal cells the rods and the arms of the Vs are straight, indicating that they are subject to some tension. Although no equatorial plate is formed in the cells, if the force or mechanism of nuclear division is the same here as it is in all other cells, then the chromosome configurations at telophase would be expected to correspond to the ordinary configurations at this stage. No such constant or expected arrangement was found. All figures showed very irregular chromosomes. The constriction points were foremost in a rather small percentage of the observed cases. In no generative cell was a complete set of chromosomes found with the constricted point preceding the remainder of each chromosome. The chromosomes were not usually straight, but formed rather irregular rods and loops which seemed to be unexplainable on a fiber separation basis. A careful examination of the shapes of the telophase chromosomes showed that they pass to the ends of the generative cell as irregular rods in a great number of cases, instead of separating as Vs (fig. 6). From the appearance of the material alone it was evident that the usual force of chromosome division at mitosis is non-operative in the generative cell. Certain other factors are present at the division of this cell, however, which are found at no other cell division in the life history of the organism. That these factors could influence the mitotic behavior seems probable. STRASBURGER and HERRIG have both reported spindle fibers in the pollen tube. NAWASHIN, however, in view of the odd shapes of the telophase chromosomes and the absence of spindle fibers in his material, has concluded that the chromosomes go to the poles by some type of movement of their own.

The two influences on the chromosome shapes in the generative cell which most probably would be of importance are the streaming of the cytoplasm of the generative cell and the alterations produced in the cell during its movement down the tube. In all ordinary tissue cells which are dividing, the achromatic figure usually forms a clear zone in the cytoplasm. In the generative cell, however, where no achromatic figure is formed, the movement of the cytoplasm might possibly affect the chromosome configurations.

The movement of the generative cell might also be responsible for

the chromosome configurations. In the spore, the shape of this cell is that of a crescent. The pollen tube places definite limitations on the shape of the generative cell, however, and consequently the form of the cell in the spore bears a remote resemblance to its form in the tube. The diameter of the tube is much too small to accommodate the generative cell and the streaming of the vegetative cell. The gametic cell in the pollen tube is long, and small in diameter, therefore, and moves down the tube as a long cylinder. The ability to change shape seems to prove that this cell is plastic and easily influenced by external conditions. If in its movement down the tube it should change shape, as a result of the streaming of the vegetative cell cytoplasm or the contour of the tube, this change would be expected to affect also the chromosome configurations, since, owing to the narrowness of the generative cell, each chromosome lies close to the cell wall and tube wall. As a result of streaming of the cytoplasm of the generative cell and of movement of the generative cell down the tube, therefore, the normal chromosome configurations at telophase could be altered to those which are actually observed.

An examination of the relative positions of the chromosomes at both ends of the generative cell seems to indicate that the time of division is not so precise at this division as it is in ordinary cells (fig. 14). The chromosomes, therefore, neither occupy a position in one plane nor undergo division at precisely the same time, as is common in most cells. These facts seem to demonstrate that the chromosome is more of an individual unit in division than has been assumed. The chromosomes in the generative cell seem, without spindle fibers, equatorial plate, and achromatic figure, to function as autonomous units. This division would seem to invalidate those theories of mitosis which postulate a simultaneous division of chromosomes lying in one plane, resulting from cytoplasmic streaming, balanced electrical and magnetic phenomena, or delicately unstable colloidal systems.

Cytological basis for inversions and translocations

The sperm nuclei which result from division of the generative nucleus seem to afford an opportunity for correlating certain genetic and cytological phenomena. These nuclei present unusual structural

relationships which can be observed clearly in late telophase stages. In somatic divisions the chromosomes seem to be arranged regularly at telophase with little or no interlocking. As they pass into the resting stage, the arrangement of the individual chromosomes can no longer be followed. In the sperm nucleus, however, each chromosome maintains its structural identity. The chromosomes may be so twisted that one segment is in contact with another segment of the same chromosome, or non-homologous chromosomes may be interlocked.

Inversions, translocations, segmental interchange, and deficiencies are commonly produced by the effect of X-rays on pollen grains of plants and sperm cells of animals. If the X-rays cause breaks in the chromosome threads which may reunite in new associations, the results may be explained on the basis of the structural relations of the chromosomes as found in the gametic nuclei. If a single chromosome forms a loop, a break at the point of contact might produce an inversion. Segmental interchange would result if two chromosomes were broken at the point of contact, followed by a reunion of non-homologous segments. It is possible, of course, that no such intimate contact of chromosomes is necessary for explaining changes in structural relationships, but the fact that such a physical relation of the chromosomes is found is at least suggestive.

Summary

1. The gametophytic development, after the formation of the mature spore, is described for *Lilium regale*.

2. Explanations for the peculiar configurations of generative cell chromosomes are presented.

3. Evidence is presented that the chromosome is more of an autosome unit at mitosis than has previously been assumed.

4. A cytological explanation of the mechanism of the formation of chromosome interchanges is presented.

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EXPLANATION OF PLATE IX

FIG. 7.—Metaphase showing the lack of equatorial plate and numerous twists in sister-threads.

FIG. 8.—Metaphase in a wider tube showing, apparently, an attempt at plate formation with constrictions in an approximate plane.

FIG. 9.—Sperm nucleus immediately after the reorganization of the telophase clump. The solid chromosome rods can be seen in place of the usual fine reticulum. This structural relationship is of possible genetic importance.

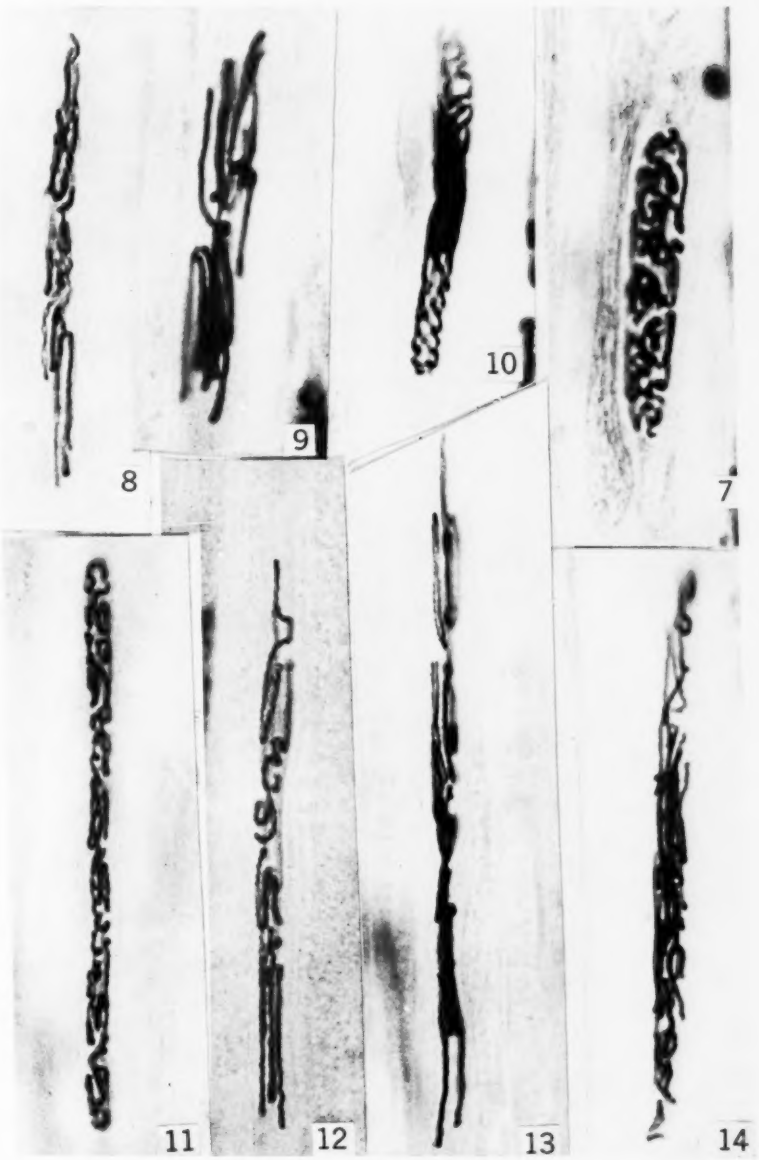
FIG. 10.—Generative nucleus with vegetative nucleus constricted against tube. The relative staining properties of these nuclei are apparent.

FIG. 11.—Usual structure of the generative nucleus at early prophase. The double threads can be seen in many places.

FIG. 12.—Metaphase involving chromosomes of the generative cell.

FIG. 13.—Metaphase of generative cell.

FIG. 14.—Anaphase of generative cell chromosomes; no equatorial plate present; chromosomes at ends of figure have obviously divided much earlier than those in the middle.



O'MARA on LILIUM

DETERMINATE CLEAVAGE POLYEMBRYONY, WITH SPECIAL REFERENCE TO DACRYDIUM

JOHN T. BUCHHOLZ

(WITH TEN FIGURES)

Introduction

A distinction may be made between the two kinds of cleavage polyembryony found in conifers: determinate and indeterminate. In the indeterminate condition there are no indications, from the appearance of the early embryo, that a particular one of the embryonic units has a distinct advantage during embryonic competition. Any one of several embryos derived from the same zygote may become the survivor. In determinate cleavage polyembryony one embryo, usually the terminal one, is more favorably situated than the others, and this is ordinarily the successful embryo. In *Dacrydium*, for example, an embryo unit found at the tip in the group of embryo initials at the end of a prosuspensor is more favorably situated, while one of those in the group immediately behind it can become functional only if the terminal embryo becomes aborted. Here we find an organization in which the position of the various embryonic units, from the earliest stages, usually determines which one is to contribute the successful embryo.

In making comparisons of the various embryogenies of conifers, it has seemed convenient to make this distinction. The description of various embryogenies is greatly facilitated by such a characterization, and the writer has become convinced that the condition of determinate cleavage polyembryony represents one of several steps in the evolution of polyembryony.

A description of the pine embryo has been given (**1**, **2**, **5**), and it will not be necessary to redescribe this type of embryogeny in illustrating indeterminate cleavage polyembryony. Any one of the four lower embryo initial cells of the 12-celled proembryo of the pine may contribute the successful embryo of the seed. In the later stages, after the primary suspensors have begun to elongate, or even in the

stages in which the four primary embryos have become completely separated from one another, one may usually recognize, for a time, a moderate degree of equality in the stage of development of the individual embryos belonging to the same system, in the number of cells or length of the suspensor, etc. At any rate, it may be stated that any one of the four embryos has a fair chance of becoming the surviving embryo or "seed germ." Similar conditions are found in *Cedrus* and in *Tsuga* (**2**, **5**).

The embryogeny of *Biota* (**4**), *Chamaecyparis* (**7**), or *Cryptomeria* (**8**) may further illustrate the indeterminate type of cleavage polyembryony. Among these it might be supposed, after considering the early embryo or proembryo, that the embryonic unit which is most nearly terminal in position is the one to be favored during embryonic selection. Among the embryos which result, however, there is usually very little difference in the stage of development, and, for a brief period at least, all or nearly all of them may develop suspensors. It appears that any one of the terminal group of embryos still has a fair chance of becoming the successful embryo of the seed, and these types may be considered as essentially indeterminate.

Whether we consider *Pinus*, *Cedrus*, *Tsuga*, or the embryos of the Cupressaceae,[1] any of the terminal embryos are favorably situated in comparison with the embryos which may sometimes develop when prosuspensor or rosette cells develop embryos. Thus all embryogenies have an element of being more or less determinate. In fact, a very sharp line of distinction cannot be drawn with certainty between the two types of cleavage polyembryony, but the important thing is that in the indeterminate type there are several embryos situated in the terminal group from which a selection is to be made during later stages of the embryogeny, after suspensors begin to appear on the individual units.

The writer has investigated embryogenies of several genera which appear to fall in the determinate class, notably *Pseudolarix* (**5**), of which only a few stages have been described. Outstanding examples of determinate cleavage polyembryony are not so well known, however, and it will serve a useful purpose to redescribe the embryogeny

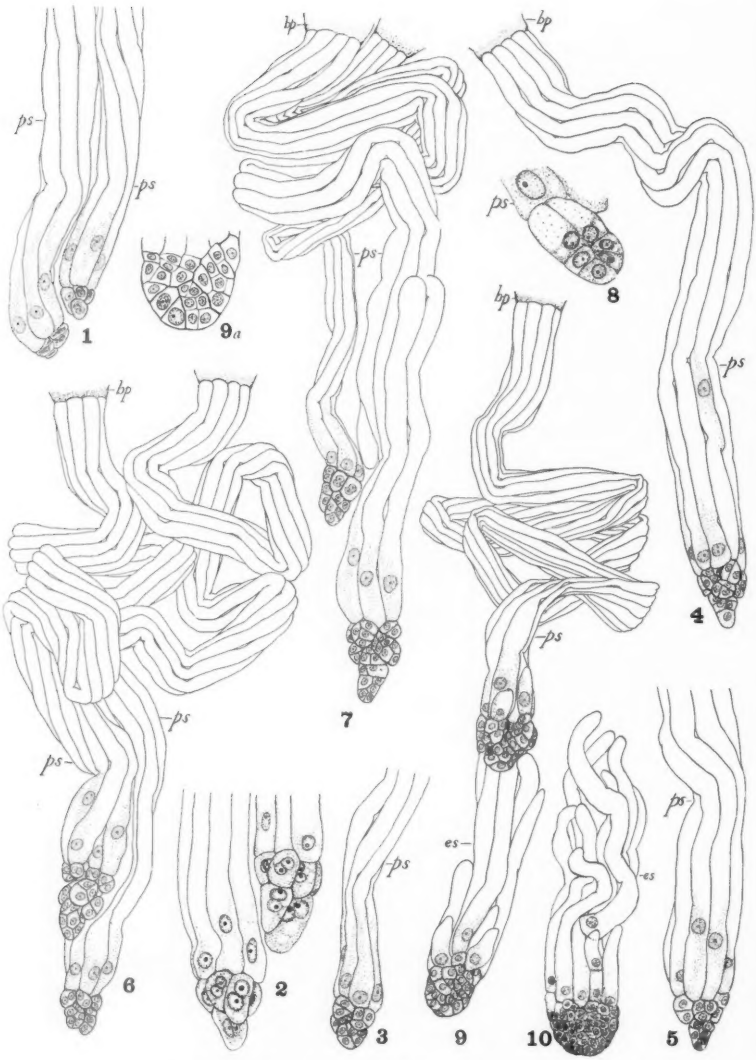
[1] Where reference is made to families in this paper, a classification (**9**) which divides the Coniferales into ten families is followed.

of *Dacrydium cupressinum*, including more details and a few stages which are supplementary or additional to the stages previously described (**4**).

Embryogeny of Dacrydium

Figures 1–10 illustrate some of the principal stages in the embryogeny of *Dacrydium cupressinum*. The proembryo is unknown. SINNOTT (**11**) gives an excellent figure of the archegonium before fertilization and many other details of the morphology. Among his figures he shows one gametophyte containing an embryo which may be in the stage of figures 3–7. Figure 1 shows two embryo systems in one of the earliest stages which was observed. The embryo system at the right shows a typical arrangement of five or six early embryonic cells situated below the prosuspensor. The one at the left bears a similar group of six or seven embryonic cells which have been crushed. The prosuspensor is usually composed of 7–9 cells. In figure 1 the prosuspensors have elongated to a length which is only slightly in excess of the part shown. (The missing upper portion was cut off in dissection.) Each of the five or more binucleate cells at the tip is an embryo initial. The arrangement of these initials varies somewhat, and sometimes a greater number of embryo initials may be found, as many as eight or nine having been observed. Figure 2 shows the lower portion of two adjacent embryo systems in about the same or a slightly earlier stage, under higher magnification. The embryo system at the left has a terminal embryo initial which has been injured and is slightly plasmolyzed. It has a total of six embryo initials, five of which are visible in the drawing. The terminal units are frequently larger than those situated at a higher level, even in this early stage. The embryo system at the right in figure 2 has eight or nine embryo initials. Embryo initials in these early stages are usually binucleate cells.

Figure 3 shows the stage which follows, after the prosuspensor has elongated to one-third or one-half its ultimate length. Each of the six or more binucleate embryo initials is now replaced by a spherical group of cells, and among these the terminal unit is considerably larger than the others. Figures 4 and 5 show similar stages, the former with seven embryonic units and the latter with six. SINNOTT, who gave some attention to the embryogeny of podocarps, un-



FIGS. 1–10.—Stages in early embryogeny of *Dacrydium cupressinum;* explanation in text. *ps*, prosuspensors; *es*, secondary suspensor; *bp*, position of plate or callose plug at upper end of prosuspensor. Figs. 1, 3–7, 9, 10, ×75; figs. 2, 8, 9*a*, ×150.

doubtedly observed these stages in his sections, for he speaks of the embryo of *Dacrydium* as invariably budding to form additional embryos.

Figure 6 consists of two embryo systems side by side, and shows the characteristic twisting and the great length to which the prosuspensor may stretch; also the manner in which it becomes coiled and twisted. The embryo system below has six distinct embryonic units; the upper one has nine. In the latter (upper system, fig. 6) the terminal unit has become large, composed of nearly a dozen cells; while a few of the units adjacent to the prosuspensor are still in the binucleate embryo-initial stage. It is obvious even in this early stage that this terminal unit will have an advantage in competition with the other embryonic units found within the same system. There is some question, however, as to whether one of the terminal embryos of the lower system (fig. 6), imbedded more deeply within the female gametophyte, will ultimately contribute the successful embryo on account of its more advantageous location, since there are only about half as many cells as in the larger embryo of the upper system.

Figure 7 shows the reverse of figure 6, in having the larger and longer embryo system the one with the greatest number of embryonic units and the shorter one with fewer units. The longer prosuspensor has eight units, the shorter six. All save the terminal embryo units of the upper system are still in the binucleate condition. The prosuspensors in the upper portion are closely coiled and interwoven, and difficult to follow. This is the usual condition of the prosuspensor when several systems are present. The upper portions of the prosuspensors are similarly interwoven but are partially loosened through dissection (fig. 6).

The embryonic units stand out more or less distinctly in all of these early stages, and in each system the terminal embryo is much larger and has more cells than the embryonic units which lie above it. The larger terminal embryo has a distinct advantage over the other units of the same system, and sometimes this may be recognized early, as in the stage shown in figure 2. It is only when this terminal embryo becomes aborted that one of the others has a chance to contribute the embryo of the seed. Thus the determinate nature of the embryo may usually be recognized at a very early stage.

In the stages which follow figures 6 and 7, the largest terminal embryo develops a secondary suspensor by the elongation of embryonal tubes from its proximal end. This massive secondary suspensor thrusts upward the units lying immediately above it, together with the prosuspensor, as shown in figure 9, where three of the four or five embryonic cell groups are shown between the prosuspensor and the secondary suspensor of the terminal embryo. It will be noted that a few of the upper embryos are also beginning to form embryonal tubes. This stage gives the appearance of a suspensor system with embryos between two sections of suspensors.

Figure 10 is a terminal embryo similar to the one in figure 9, in a slightly later stage, the oldest stage which was available. The embryo is still undifferentiated except for the polarity shown by the two ends. It consists of a spherical mass of cells with embryonal tubes elongating from the proximal end to form a massive secondary suspensor, but in this stage there are still no traces of stem tip or cotyledonary primordia.

A large deposit (*bp*) forming a plate or plug, which COKER (**10**) described as a callose plug in *Podocarpus*, is usually found immediately above the prosuspensor. In some preparations this plug was lost in dissection; in others it was present in the positions which are shaded in the drawings. If rosette cells or rosette embryos are found in other conifers, this plate or plug is found above the rosette group.

No rosette cells were observed. These cells or their remains should be found above the prosuspensor (**4**). If rosette cells are formed here, it is certain that they are very ephemeral and leave no trace in embryo systems of the stage shown in figure 4.

A careful examination of the embryo of figure 10 gave no indication of an apical initial cell. The embryo of figure 9 still has an apical cell, however, which may be identified at its tip in a deep plane of focus. A view of its optical section in a median focus is shown under greater magnification in figure 9*a;* figure 9, a surface view of the cells, shows only a small portion of the apical cell. Figure 8 is an embryo of 10–12 cells with its apical cell shown in a median plane of focus still in contact with the prosuspensor. In this case the embryonal tubes are just beginning to elongate.

Nearly all of the individual embryos in the different groups (figs. 3–9) give the appearance of having the apical-cell method of growth.

The apical cell, therefore, appears from the time of wall formation in the various embryo initials, and persists at least until an embryo of the stage of figure 9, comprising approximately 75–80 cells, has developed. It disappears completely before an embryo of twice this size (3–4 times as many cells) has been formed.

Aside from showing the apical cell and determinate cleavage polyembryony, there is still another feature in the embryogeny of *Dacrydium* which is of special interest. There is no primary suspensor; that is, a single elongating cell from each embryo which precedes the formation of embryonal tubes. *Cryptomeria* (**8**) has no primary suspensor; while *Sciadopitys* (**6**), *Biota* (**4**), and *Chamaecyparis* (**7**), as well as *Pinus* (**5**), all have primary suspensors, although strictly speaking the last may be considered as having no prosuspensor. In *D. cupressinum* the individual embryo developing at the end of a prosuspensor produces, from the beginning, only a secondary suspensor composed of many embryonal tubes.

Discussion

The embryogeny which has been described for *Dacrydium cupressinum* was previously given in its general outline (**4**). Very little was stated concerning the apical cell growth of the early embryo, and since not all of the available material had been examined when the scheme of embryogeny was first described, the writer has felt justified, after a more detailed study, in presenting this embryogeny in a more complete form. It may be pointed out that the individual embryos omit the formation of a primary suspensor. This distinction was not made until the embryogenies, and especially the suspensor systems, of *Cryptomeria* (**8**) and certain Cupressaceae (**4, 7**) had been carefully investigated and compared. The omission of the primary suspensor need not be taken to suggest affinities between *Dacrydium* and *Cryptomeria*, but should be looked upon as due to parallelism in the evolution of embryogenies within each of two distinct phylogenetic lines. There appear to be other families of conifers among which this distinction may be made. The presence of the primary suspensor (a single-celled elongating cell) may be regarded as a more primitive condition; the omission of this part as a more advanced condition.

The distinction between determinate and indeterminate cleavage

polyembryony may be made for the embryogenies of Pinaceae (Abietineae) (9), where *Pseudolarix* (5) represents the determinate type of cleavage polyembryony, as well as for the Podocarpaceae (9). While the type of embryogeny of *Pseudolarix* has only been hinted at thus far (5), it seems obvious that, on the basis of polyembryony, this type belongs in the same class, or on the same level, as that of *Dacrydium*.

The probable steps in the evolution of polyembryony may be regarded as follows: (*a*) indeterminate cleavage polyembryony; (*b*) determinate cleavage polyembryony; (*c*) simple polyembryony showing definite vestigial traces of determinate cleavage polyembryony; (*d*) simple polyembryony without traces of determinate cleavage polyembryony. Among the vestigial traces are rosette embryos and rosette cells, or the individual prosuspensor cells which may break away from the group and produce embryonic cells on their tips, as found in both *Cryptomeria* (8) and *Chamaecyparis* (7).

The embryogeny of *Cephalotaxus* (3) is very interesting in the light of these distinctions. There is no apical cell and no primary suspensor. The terminal embryo initials on the end of a prosuspensor all combine to form a single embryo, save for the units represented by the cap cells which become dead cells and break off at an early stage. Above the prosuspensor is a group of rosette cells each of which develops more or less as an independent embryo. In a sense this type may be considered as representing a form of cleavage polyembryony, but as such it is decidedly of the determinate type. The embryogeny would fall into a higher category than that of *Dacrydium*. The latter represents the second (*b*) category, while *Cephalotaxus* falls more nearly into the (*c*) category; so much so that the embryogeny has been described as essentially representing simple polyembryony.

Only a few comparisons have been pointed out, but these are probably sufficient to illustrate the value of the distinction between the determinate and indeterminate types of cleavage polyembryony. To include a discussion of the embryogenies of all types which have been observed does not seem necessary at present.

A brief diagrammatic summary of some of the embryonic characteristics, together with the diagnostic taxonomic characters of the families of conifers, is given elsewhere (9).

Summary

1. A distinction may be made between the two types of cleavage polyembryony: indeterminate and determinate.

2. The embryogenies of *Pinus*, *Cedrus*, *Tsuga*, *Cryptomeria*, *Biota*, and *Chamaecyparis* illustrate the indeterminate type of cleavage polyembryony.

3. The embryogeny of *Dacrydium cupressinum* is described in some detail and used to illustrate an embryogeny with determinate cleavage polyembryony.

4. A prosuspensor but no primary suspensors are found in *Dacrydium*.

5. No traces of rosette cells were observed in *Dacrydium*.

6. The early embryo system of *Dacrydium* may be recognized as being compound or composite through binucleate embryo initial cells borne on the prosuspensor.

7. The individual embryos of *Dacrydium* were found to develop by apical cell growth, beginning with the formation of walls in the initial cells and continuing to a stage of more than 75–80 cells; but an apical cell was not found in embryos of three or four times this number of cells.

8. The embryogenies of conifers may be separated into at least four levels with reference to indeterminate and determinate cleavage polyembryony, and simple polyembryony with or without vestigial cleavage polyembryony.

9. These new distinctions in types of cleavage polyembryony or simple polyembryony facilitate the comparison of embryogenies.

The writer is greatly indebted to Professor E. W. SINNOTT for supplying the material of *Dacrydium cupressinum* used in this investigation. The collections were made at Woodlands, New Zealand, in 1911. The younger stages were killed on January 13 and the older stages on January 22.

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. VIII

EARL EDWARD SHERFF

BIDENS PERSONANS Degener & Sherff, Bot. Gaz. **92**:205. 1931.—The type of this species was *William Bush* 32, alt. 1200 feet on south slopes in semiarid valley, Palikea, Isl. Oahu, Jul. 7, 1929. It was given its trivial name in allusion to the diverse characters displayed, these suggesting no fewer than three distinct species: *Bidens fulvescens* Sherff, *B. sandvicensis* Less., and *B. conjuncta* Sherff. More recently a further specimen, collected at the same time and place (*Bush* 33), has been sent me by Professor OTTO DEGENER. This has the exact foliar habit of *B. fulvescens* instead of *B. personans*, but resembles the latter in the fact that the immature achenes are but slightly twisted or looped. From my studies upon several cases of hybridity brought to light in the recent collections of Professor DEGENER and his associates, it appears probable that *B. personans* will be found to be a hybrid of *B. fulvescens* with some other species.

Bidens halawana Degener & Sherff, sp. nov.—Planta inferne fruticosa superne herbacea, erecta, glabra, 0.9–3 m. alta, ramis angulatis. Folia petiolata petiolis tenuibus saepuis 2–5 cm. longis, petiolo adjecto saepius 7–15 cm. longa, nunc simplicia lamina membranacea ovata moderate grosseve acridentata apice acuta vel subacuminata, nunc 2–3-partita foliolis membranaceis ovatis vel ovato-lanceolatis acriter serratis vel interdum integris lateralibus plus minusve sessilibus. Capitula paniculato-corymbosa, tenuiter pedicellata pedicellis glabris plerumque 1–2 cm. longis, radiata, pansa ad anthesin circ. 6–8 mm. alta et circ. 1–1.6 cm. lata. Involucri bracteae exteriores 4 vel 5 (rarius 3–6) lineari-oblongae, apice subobtusae, glabrae, 1–2.7 mm. longae, quam interiores oblongae vel oblongo-ovatae apice saepe pubescentes plerumque dimidio breviores. Flores ligulati 3–5, flavi, ligula ovato-oblongi vel oblongo-elliptici, apice dentati, 6–8 mm. longi. Paleae demum linearioblongae et marginibus hyalinae, concavae, 5–8 mm. longae, in-

volutae et achaenia subtendentes. Achaenia plano-obcompressa, faciebus marginibusque glabra, nigra, exalata, corpore (moderate arcuato vel torto, raro per 1–2 revolutiones voluto) 8–12 mm. longa et 1–1.7 mm. lata, ad apicem vel raro paululum infra apicem biaristata aristis tenuibus parce retrorso-hamosis ∓1 mm. longis.

Specimens examined: *Otto Degener, Kwan Kee Park, Yoshimasa Nitta,* and *William Bush* (*Degener distrib. no.*) 4133, on partly forested lateral ridge, north slope of South Halawa Gulch, about one mile from top of Koolau Range, Isl. Oahu, Hawaiian Isls., Apr. 17, 1932 (type, Herb. Field Mus., 2 sheets; cotypes, Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.).

Bidens ferax Degener & Sherff, sp. nov.—Fruticosa, erecta, glabra, ramosa, ∓1m. alta, ramis tetragonis. Folia opposita, petiolata petiolis tenuibus circ. 2–5 cm. longis, petiolo adjecto circ. 6–13 cm. longa, simplicia vel saepissime tripartita, foliolis lanceolatis vel ovato-lanceolatis, membranaceis vel subcoriaceis, serratis, acuminatis, lateralibus nunc sessilibus nunc tenuiter petiolulatis. Capitula numerosissima, dense corymboso-paniculata pedicellis tenuibus parce glabratis ∓1 cm. longis, pansa ad anthesin circ. 1.5–1.8 cm. lata et 5–7 mm. alta. Involucri bracteae exteriores circ. 4 vel 5, patentes vel reflexae, lineari-oblongae, glabratae vel hispidulae, apice acutae vel obtusae, 1–1.5 mm. longae; interiores oblongae 4–6 mm. longae. Flores ligulati 4–6, flavi, ligula elliptico-oblongi vel cuneato-obovati, apice valde 2- (raro 3-) dentati, 7–9 mm. longae. Achaenia oblongo-linearia, valde obcompressa, non vere alata, nigra, unaquaque facierum circ. 4-sulcata, faciebus marginibusque glaberrima, corpore circ. 8–11 mm. longa et circ. 0.5–0.8 mm. lata, supra (vix infra apicem ipsum) sparsim setosa et biaristata aristis tenuibus retrorsum hamosis 0.4–1 mm. longis.

Specimens examined: *Otto Degener, Kwan Kee Park, Yoshimasa Nitta,* and *Philip Westgate* (*Degener distrib. no.*) 4117, dry, grassy slope, ¼ mile from shore on south side of Keaau Valley, Isl. Oahu, Hawaiian Isls., Mar. 23, 1932 (Herb. Field Mus., 2 type sheets; Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mun.).

Bidens aspilioides (Baker) comb. nov.; *Coreopsis aspilioides* Baker, Kew Bull. **1898**:153. 1898.—An African species from the region to the south of Lake Nyassa. The type was collected by *Alexander Whyte* and should be at Kew or in the British Museum

of Natural History, but repeated searches in both of these places as well as elsewhere have failed to locate it. The leaves are lanceolate, scabrous, and entire or scarcely dentate. The achenes are glabrous except for the two small, lanceolate pappus awns, and are presumably exalate.

Bidens articulata nom. nov.; *Coreopsis glaucescens* O. & H. *in* Oliv. Fl. Trop. Afr. **3**:389. 1877; *C. abyssinica* f. *latisecta* Vatke, Linnaea **39**:499. 1875 (*nom. subnudum*).—Belonging in the section *Steppia*, where it stands closest to *B. rotata* and *B. vatkei*. From *B. rotata* (with achenial bodies 4–6 mm. long and leaves 3-partite) it differs in having achenial bodies 3.5–4.5 mm. long and leaves 3–5-partite, etc. From *B. vatkei* (with achenial bodies 3–4 mm. long, exterior involucral bracts about 6–7 mm. long, leaves slenderly petioled) it differs in having achenial bodies averaging slightly larger, exterior involucral bracts 1.3–1.7 cm. long, leaves subsessile or petiolate with flat, margined petioles, etc.

Bidens acrifolia sp. nov.—Herba forsitan perennis, plus minusve glabrata, caulibus vel ramis gracilibus. Folia opposita, petiolata petiolis usque ad circ. 1 cm. longis, petiolo adjecto principalia saltem 3–4 cm. longa, bipinnatisecta segmentis linearibus firmulis terminaliter subsensim ad apicem acerrimum angustatis, 1–3 mm. latis; summa simplicia lineariaque vel unipinnata. Capitula solitaria tenuiter pedunculata pedunculis ∓5 cm. longis ramum terminantibus, radiata, pansa ad anthesin ∓2.5 cm. lata et ∓8 mm. alta. Involucri plus minusve hispiduli bracteae exteriores circ. 12, lineares, acutae, 5–7 mm. longae, quam interiores ovato-oblongae paulo longiores. Flores ligulati ∓6, flavi, ligula elliptico-oblongi, circ. 1–1.4 cm. longi. Achaenia matura deficientia. Ovaria minima, plana, biaristata aristis tenuibus calvisque.

Specimens examined: *M. P. Dehesa* 1532, alt. 1600 m., Mala Noche, Concordia, State of Sinaloa, Mexico, September (type, Kew; *nom. vulg.* palmita; *unico ramo viso*).

The general habit, so far as it can be inferred from the single branch examined, is somewhat similar to that of *Bidens geraniifolia* Brandeg. and *B. triplinervia* var. *macrantha* (Wedd.) Sherff. The foliar segments are especially acute, however, and the unbarbed achenial aristae should render distinction easy.

Bidens amplectens Sherff × **B. waianensis** Sherff, hybr. nov.—*B. waianensi* achaeniis supra medium valde recurvatis vel subtortis similis, aliter *B. amplectenti* plus minusve similis.

Specimens examined: *Otto Degener* and *D. LeRoy Topping* (*Degener distrib. no.*) 4120, on lantana-covered, somewhat dry slope, along trail leading to top of Keawaula Valley, Isl. Oahu, Hawaiian Isls., Mar. 24, 1932 (Herb. Berl.; Herb. Brit.; Herb. Field Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.).

Recently Professor OTTO DEGENER forwarded me some specimens collected by himself and Mr. D. LEROY TOPPING along the trail leading to the top of Keawaula Valley, on the Island of Oahu, Hawaiian Islands. One set, numbered by DEGENER 4119, was *B. waianensis*. Specimens are being sent to several herbaria (Herb. Field Mus.; Herb. Gray; Herb. Kew). Another set, numbered by DEGENER 4121, was *B. amplectens*. Specimens of this likewise are being distributed to certain herbaria (Herb. Berl.; Herb. Brit. Mus.; Herb. Field Mus.; Herb. Gray; Herb. Kew). A third set, numbered by DEGENER 4120, was found to be of hybrid material, embodying very emphatically throughout the general habit of *B. amplectens* but approaching *B. waianensis* in the curvature or even the twisting of the distal half of the achenes.

COREOPSIS LEAVENWORTHII **curtissii** var. nov.—Folia 1–2-pinnata; basalia pinnatim circ. 9-partita, foliolis oblongo-linearibus ad basim sensim angustatis plerumque 2–4 cm. longis et 2–5 mm. latis, lateralibus (praecipue inferioribus) utrinque saepe rursus 1-lobatis lobo parvo linearique; caulina quam internodia nunc multo longiora nunc multo breviora, plerumque 2-pinnata foliolis lateralibus saepius 3 vel 4 jugis, segmentis ultimis omnibus 0.5–2.5 cm. longis et circ. 1–3 mm. latis.

Specimens examined: *Allen Hiram Curtiss* 6734, in dry, pastured ground along the Suwannee River, near Branford, Florida, October 23, 1900 (Herb. Gray, 2 type sheets).

COREOPSIS LEAVENWORTHII var. **lewtonii** (Small), comb. nov.; *Coreopsis lewtonii* Small, Bull. Torr. Bot. Club **25**:146. 1898.—E specie foliis simplicibus plerumque quam internodiis brevioribus radice saepe perenni differt.

Specimens examined: *Liberty Hyde Bailey* and *Ethel Zoe Bailey* 13047, sands at Sarasota, Florida, Sept. 28, 1929 (Herb. Field Mus.); *Mr. A. H. Curtiss* 1480, swamps and shores, Halifax River, Florida, June (Herb. Berl.; Herb. Field Mus., 2 sheets; Herb. Gray); *Mr. A. A. Eaton* 469, Little River, Dade County, Florida, Dec. 5, 1903 (Herb. Field Mus., 2 sheets); *Albert S. Hitchcock* 139, around flatwood ponds, Myers, Florida, July–August, 1900 (Herb. Field Mus.); *ex herb. S. C. Hood*, low flatwoods, Deep Creek, Florida, June 6, 1910 (Herb. Mo. Bot. Gard.); *Frederick L. Lewton*, Forest City, Florida, 1894 (type, Herb. N.Y. Bot. Gard.); *idem*, Cedar Hammock, Sumter County, Florida, Aug. 3, 1894 (Herb. N.Y. Bot. Gard.; *George V. Nash* 2255, Sanford, Florida, Jul. 24, 1895 (Herb. Berl.; Herb. Field Mus.; Herb. Gray); *Mr. F. Rugel* 133, Florida, 1843 (Herb. Gray; Herb. Mo. Bot. Gard., 2 sheets); *J. K. Small* and *J. J. Carter* 3025, the Everglades, intersecting Long Key, Florida, Jan. 18–26, 1909 (N.Y.); *John Donnell Smith*, pine-barren ponds, Pellicier's Creek, St. John's County, Florida, Mar. 4, 1882 (Herb. Univ. Chi.; Herb. Field Mus., 2 sheets; Herb. Gray); *Mr. S. M. Tracy* 7256, Long Key, Florida, May 8, 1901 (Herb. Berl.; Herb. Field Mus.; Herb. Gray); *idem* 7355, Perico Isl., Dec. 6, 1901 (Herb. Gray; Herb. Mo. Bot. Gard.).

An examination of the foregoing specimens in comparison with a great number of specimens of *C. leavenworthii* proper fails to show more than mere varietal differences. Upon reducing *C. lewtonii* to varietal status under *C. leavenworthii*, we have a total of three varieties for that species: var. *garberi* A. Gray, var. *curtissii*, and var. *lewtonii*. These may be distinguished by the following key:

Folia simplicia.....var. δ. *lewtonii*
Folia principalia 1–2-pinnata
  Foliorum caulinorum foliola terminalia nunc obovata nunc lanceolato-oblonga, 0.8–1.5 cm. lata.....var. β. *garberi*
  Folia caulinorum foliola terminalia (vel eorum segmenta ultima) circ. 1–3 mm. lata
    Folia basalia pinnatim circ. 9-partita.....var. γ. *curtissii*
    Folia basalia integra.....*C. leavenworthii* sensu stricto

COREOPSIS GRANDIFLORA var. **harveyana** (A. Gray), comb. nov.; *Coreopsis harveyana* A. Gray Synopt. Fl. N. Amer. 1[II]: 292. 1884.—Foliorum segmentis anguste linearibus vel quidem filiformibus, achaeniis paulo minoribus circ. 2 mm. longis differt.

Specimens examined: *Anon.*, Arkansas National Forest, Arkansas, 1909 (Herb. U.S.); *Mr. B. F. Bush* 158, Sheffield, Missouri, June 12, 1894 (Herb.

Berl.); *Frederick V. Coville* 127 Ark., Mountain Park near Little Rock, Arkansas, Jul. 17, 1887 (Herb. U.S.); *Mr. H. Eggert*, Williamsville, Missouri, June 11, 1893 (Herb. Field Mus.); *Prof. Francis LeRoy Harvey*, cliffs near Fort Smith, Arkansas, June (Herb. Gray, type, *cum C. tinctoria* Nutt. *pro parte parva commixta*); *Mr. E. J. Palmer* 8120, wet, open hillsides, Malvern, Arkansas, June 23, 1915 (Herb. Mo. Bot. Gard.); *H. S. Reynolds*, Judsonia, Arkansas, May 31, 1877 (Herb. Field Mus.); *Huron H. Smith* 1150, railway tracks, Des Arc, Iron County, Missouri, June 8, 1908 (Herb. Field Mus.).

The type sheet of *C. harveyana* A. Gray bears three specimens. The first at the left is *C. tinctoria* Nutt., having the oblong wingless achenes, the bicolored ligules, the subtruncate style-branch tips, the linear-oblong paleae, etc. of that species. The other two are very distinct, having orbiculate and winged achenes, caudate-conical tips to the style branches, upwardly elongate-attenuate paleae, etc. GRAY clearly was misled by the admixture of the small *C. tinctoria* specimen, for in his Synoptical Flora he separated *C. harveyana* partly on the basis of the "rays sometimes brown-purple at base." Had the rays of *C. harveyana* really possessed the tendency toward a bicolored state, a separation of the type as a new species would have been justified. Since the rays were really yellow, however, the type had nothing left to separate it from *C. grandiflora* proper except the narrower leaf divisions and the slightly smaller achenes. These differences GRAY himself elsewhere (*e.g.*, *C. drummondii* Torr. & Gr. *et* var. *wrightii* A. Gray Synopt. Fl. N. Amer. 1[II]: 291. 1884) considered as connoting a variety.[1]

COREOPSIS TINCTORIA **imminuta** var. nov.—Capitula pansa ad anthesin 1–1.5 cm. lata, bracteis exterioribus ∓1 mm. interioribus circ. 5–6 mm. longis, achaeniis 0.9–1.4 mm. longis.

Specimens examined: *John Michael Holzinger*, Santa Fe, New Mexico, August 13, 1911 (type, Herb. Mo. Bot. Gard.).

Coreopsis killipii sp. nov.—Fruticosa, glabra, 6–12 dm. alta, ramosa ramis tenuibus angulatis internodiis quam foliis longioribus. Folia petiolata petiolis moderate ciliatis circ. 4–13 mm. longis basi in poculum ∓1.5 mm. altum connatis, petiolo adjecto circ. 1.5–2.5 cm. longa, pinnatim vel subbipinnatim divisa, segmentis ultimis saepius oblongo-linearibus crassiusculis eciliatis planis vel vix revolu-

[1] A supposedly duplicate sheet of the *Harvey* material at St. Louis (Herb. Mo. Bot. Gard.) bears all *C. tinctoria* Nutt.

tis apice acutis subacutisve. Capitula pauca, in pedunculis ramos elongatos superne nudatos terminantibus ∓1–1.2 cm. longis disposita, radiata, pansa ad anthesin ∓2.6–2.8 cm. lata et ∓0.8–1 cm. alta. Involucrum tantum ad basim ipsam hispidum, bracteis exterioribus circ. 8, oblongis, eciliatis, apice obtusis vel rotundatis, 3–5 mm. longis et 1–1.7 mm. latis; interioribus ovato-oblongis, siccis plus minusve atris, apicem versus vix ciliatis, 7–10 mm. longis. Flores ligulati forsitan 5 vel 6, brunneo-flavi, ligula elliptico-oblongi, apice integri vel parce denticulati, circ. 1.2 cm. longi. Paleae spathulato-oblanceolatae, dorso (praecipue ad costam medianam) hispidae ventre glaberrimae, apicem acutum versus ciliatae, circ. 5–6 mm. longae ad corpus achaenii strictim applicatae. Flores tubulosi subbrunneo-flavi, stylorum ramis incrassatis apice subito breviterque conico-appendiculatis. Achaenia valde obcompressa, cuneato-oblanceolata, nigra vel fere purpurascenti-nigra, dorso glaberrima, ventre marginibusque perspicue erecto-pilosa pilis elongatis sericeis, corpore 4–5 mm. longa, apice biaristata aristis linearibus stramineis antrorsum hispidis 1.5–2.5 mm. longis.

Specimens examined: *Ellsworth P. Killip* and *Albert C. Smith* 21823, straggling shrub 2–4 ft. high, open hillside, alt. 3000–3200 m., Tarma, Department of Junín, Peru, Apr. 20–22, 1929 (type, Herb. Field Mus.).

The South American species of *Coreopsis* are shrubby and fall into the section *Pseudo-Agarista* A. Gray. Some (*e.g.*, *C. pickeringii* A. Gray) have comparatively large heads and light yellow rays, others (*e.g.*, *C. macbridei* Sherff) have comparatively small heads and rays which at least in the dried state are a brownish yellow. In these latter the involucres at least of the dried heads tend more conspicuously to be blackish. One such species is *C. killipii*. It is perhaps most closely related to *C. macbridei* Sherff and *C. notha* Blake & Sherff. From the former it differs at once in having smaller leaves, fewer and much larger heads, also comparatively (and actually) much larger exterior involucral bracts, etc. From the latter it differs in having smaller leaves, linear-oblong (not lanceolate to oblong) leaf segments, about 8 (not 6 exterior involucral bracts, these 3–5 (not 2–3) mm. long, etc.

Coreopsis nodosa sp. nov.—Fruticosa, forsitan 6–8 dm. alta, ramosissima ramulis nunc subteretibus nunc valde angulatis glandu-

loso-puberulis internodiis numerosissimis plerumque 1–4 rarius usque ad ∓10 mm. longis et circ. 1–1.5 mm. crassis. Folia numerosissima opposita petiolata petiolis plus minusve hispido-ciliatis basi in poculum minutum connatis 1–5 mm. longis (parte inferiore ad ramulos juncta manente post casum foliorum), petiolo adjecto tantum circ. 7–9.5 mm. longa, glabra, pinnatim 3–7-partita segmentis crassiusculis oblongis vel ovato-oblongis sparsim hispidulis apice subobtuso mucronatis plerumque tantum 2–3.5 mm. longis et 0.4–0.7 mm. latis. Capitula tenuiter pedunculata pedunculis (supra summa folia) circ. 1 cm. longis, radiata, pansa ad anthesin ∓1.8 cm. lata et 7–8 mm. alta. Involucri basaliter tomentuloso-hispidi bracteae exteriores circ. 8, cuneato-oblongae, longitudinaliter 3-vittatae, glabratae, apice obtusissimae vel rotundatae, 2–3 mm. longae; interiores cuneato-oblongae apice obtusissimae 4–7 mm. longae. Flores ligulati 6–8, claro-flavi, ligula elliptico-oblongi, apice integri ∓8 mm. longi. Paleae lineari-oblongae, dorso marginibusque erecto-hispidae ventre glabratae, apice acutae, 5–6 mm. longae, ad corpus achaenii strictim applicatae. Achaenia valde obcompressa, cuneate lineari-oblonga, atra, duabus faciebus praesertim ventrali ac marginibus valde erecto-setosa setis longis albidis sericeis, corpore circ. 4 mm. longa, apice biaristata aristis tenuibus stramineis antrorsum setosis 1–1.8 mm. longis, inter aristas poculo minuto coronata.

Specimens examined: *Francis W. Pennell* 13646, open, rocky slope, alt. 2900–3100 m., Ollantaitambo, Department of Cuzco (Cusco), Peru, Apr. 26, 1925 (type, Herb. Field Mus.).

COREOPSIS DELPHINIFOLIA **chlooidea** var. nov.—E specie foliorum segmentis magna ex parte tantum 0.3–2 (nonnullis usque ad 3.2) mm. latis differt.

Specimens examined: *Samuel Bottsford Buckley*, in pine woods, mountains of Alabama (type, Herb. Gray); *Roland M. Harper* 545, dry pine barrens, Sumter County, Georgia, Aug. 31, 1900 (Herb. Field Mus.).

The plants examined are seen from their caudate-tipped style branches and other characteristic parts to be unmistakably nearest to *C. delphinifolia* Lam. From that species they differ, however, in having the leaf segments narrower by half. It is interesting to note that in the closely related *C. major* Walt. two varieties are dis-

tinguished by their comparably narrower leaf segments, var. *linearis* Small and var. *rigida* (Nutt.) F. E. Boynt.

Coreopsis corninsularis sp. nov.—Herba perennis, erecta, gracilior, caulibus tenuibus angulatis glabris 2 vel 3 ex unico basi circ. 3–4 dm. altis. Folia opposita, plus minusve petiolata petiolis tenuibus usque ad 3 cm. longis basi sparsim hispido-ciliatis, petiolo adjecto plerumque 3–8 cm. longa et 1–6 mm. lata, saepius integra anguste vel late spathulato-linearia crassiuscula faciebus glabra marginibus ciliata apice subacuta vel subobtusa, interdum 3–5-partita foliolis lateralibus multo minoribus et tantum 1–2 mm. latis. Capitula solitaria longe pedunculata pedunculis tenuibus glabris 1–1.8 dm. longis, radiata, pansa ad anthesin 3–5 cm. lata et 10–12 mm. alta. Involucri glabri bracteae exteriores 8–10, lanceolatae vel oblongo-lineares, 3–5 mm. longae; interiores ovato-oblongae, demum circ. 1 cm. longae. Flores ligulati circ. 8, flavi, ligula oblongo-oblanceolati, apice saepius 3-lobati lobo mediano multo majore apice rursus in 2 dentes inciso, 1.5–2.5 cm. longi. Paleae tenuiter lineares, superne filiformes, 3–4 mm. longae. Flores tubulosi flavi, styli ramis terminaliter caudato-appendiculatis. Achaenia valde obcompressa, dorso convexa, nigra, corpore ipso oblongo 2.5–3.2 mm. longa et (alis exclusis) 1.2–1.5 mm. lata, faciebus glabra vel glabrata, marginibus alata alis expansis membranaceis quam corpore saltem 0.5–0.75 angustioribus, apice papposa 2 fimbriatis squamellis circ. 0.1–0.2 mm. longis.

Specimens examined: *Samuel Mills Tracy* 8542, Horn Island, Mississippi, May 25, 1903 (type, Herb. Mo. Bot. Gard.; cotype, Herb. Field Mus.).

This is an apparently endemic species from a small island in the Gulf of Mexico. It is close in technical characters to *Coreopsis lanceolata* L., from which it differs in its smaller and more delicate leaves, also its smaller external involucral bracts. The habit is strongly suggestive of *C. debilis* Sherff, found on the mainland proper of the southeastern United States, but the fewer and much larger heads, the much longer peduncles, the larger achenes, etc. all separate it from that species.

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VASCULAR ANATOMY OF THE TRANSITION REGION OF CERTAIN SOLANACEOUS PLANTS

ALBERT F. THIEL

(WITH NINE FIGURES)

Introduction

In a recent paper on the anatomy of the primary axis of the egg-plant, *Solanum melongena*, the writer (3) reported the results of his studies on root-stem transition. Since the method of transition in the egg-plant differed from that of the potato, as reported by ARTSCHWAGER (1), additional studies were made on six other species of solanaceous plants, including the potato. It was the purpose of this investigation to determine whether or not variations in the method of root-stem transition are common among species of the Solanaceae.

ARTSCHWAGER (1) was the first to give a detailed description of the root-stem transition in the potato plant. He states: "In the change from the exarch to the endarch condition it is noticed first, that the two protoxylem groups of a diarch root begin to swing outward, one following a left, the other a right curve" (fig. 1). At a point just below the cotyledons he found that the primary xylem groups, instead of forming a radial row, come to lie in a tangential plane. The change from the exarch to the endarch condition was completed in the region above the cotyledons.

KING (2), studying *Lycopersicum esculentum*, found that the primary xylem plate bifurcated and differentiated into two distinct bundles. The metaxylem differentiated tangentially toward the periphery of the stele at successively higher levels. The protoxylem maintained its original position until the level just below the cotyledonary plate was reached. Centripetal differentiation of the protoxylem began at this point and continued until the primary xylem groups were almost endarch at the cotyledonary plate.

THIEL (3), in studying the anatomy of the primary axis of the egg-plant, found that the method of transition agrees with that of KING for the tomato, but disagrees with the results of ARTSCHWAGER

for the potato plant. KING did not investigate the changes occurring in the petiole or midrib of the cotyledon. The first change in transition in the egg-plant was a breaking up of the diarch xylem plate and the two primary phloem groups, forming two units of the primary xylem and phloem. At a higher level there is a bifurcation of the metaxylem and an inward differentiation of two of the phloem groups. Near the cotyledonary plate there is a separation of the two primary

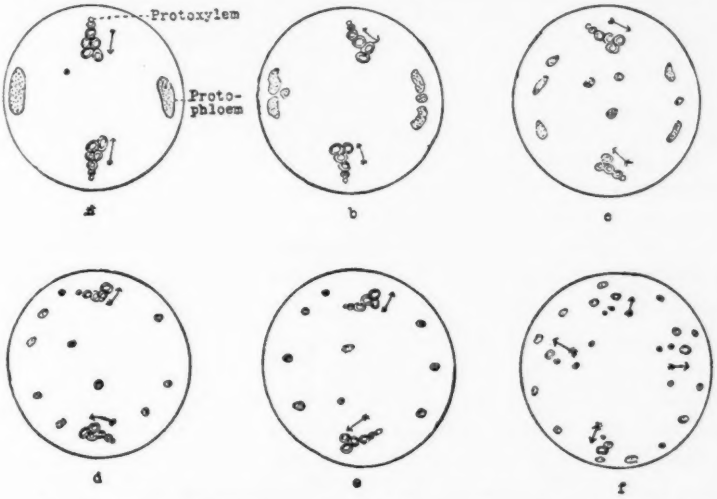


FIG. 1.—Photograph of ARTSCHWAGER'S (1) diagrams illustrating root-stem transition in the potato plant; reproduced by permission of M. C. MERRILL.

xylem units formed by the breaking of the original diarch xylem plate. One of these units becomes the vascular trace of one of the cotyledons, and the second unit, that of the other. By inward differentiation and further division, several small primary phloem groups finally come to lie opposite the inner faces of the primary xylem elements. The four remaining groups lying nearest to the metaxylem are gradually inclined in a tangential direction, eventually lying on the outside of the original position of the protoxylem points. This development continues until the bicollateral condition is established. The endarch condition is not attained in the hypocotyl but in the midrib of the cotyledon. The behavior of the primary phloem in the

egg-plant agrees in general with the findings of ARTSCHWAGER for the potato and with KING for the tomato.

MATERIAL AND METHODS.—The solanaceous plants used in this study were: potato, *Solanum tuberosum;* tomato, *Lycopersicum esculentum;* Jimson weed, *Datura tatula;* ground cherry, *Physalis virginiana; Petunia acuminata;* and Jerusalem cherry, *Solanum pseudocapsicum.*

Seedlings, varying in age from 5 to 15 days, were used for each species investigated. Studies were made with plants in which the primary tissues were just beginning to differentiate from the procambium to fully matured primary permanent tissues.

Flemming's weaker killing solution was used, and the usual laboratory methods of dehydrating and imbedding in paraffin were followed. The sections were stained with safranin and light green.

Root-stem transition

The primary root of the potato and the other five solanaceous plants investigated is a diarch, radial protostele (fig. 2). The method of transition was found to be exactly the same in each of them, and is the same type as that previously reported for the egg-plant. Since the writer's findings for the potato do not agree with those of ARTSCHWAGER, illustrations of the potato will be used in this paper, representing the typical method of transition for each plant studied.

The first change in the transition from the diarch, radial protostele of the root to the bicollateral type of bundles in the cotyledons and stem is a breaking up of the diarch xylem plate and a division of the two primary phloem groups each into three distinct groups (figs. 2, 3). This is followed by a bifurcation of the metaxylem of the two primary xylem units (fig. 4). ARTSCHWAGER's illustrations show that the two primary xylem units do not bifurcate but swing outward, one following a left and the other a right curve (fig. 1). No such condition was observed in the serial sections of many seedlings of different ages studied.

The central group of phloem cells at each side differentiates toward the center of the axis and becomes the inner phloem (figs. 3, 4). Each of the four remaining groups of the primary phloem is gradually inclined in a tangential direction toward the position of the

protoxylem points (figs. 3–5). Just below the cotyledonary plate there is a gradual separation of the two primary xylem units formed by the breaking of the original diarch xylem plate (fig. 5). One of these units, consisting of one protoxylem point, its metaxylem, and the internal and outer phloem groups, becomes the vascular trace of one of the cotyledons; and the second unit, that of the other (fig. 6). THOMAS (**4**) applies the term "double bundle" to these individual primary xylem units. Their double characteristic is very conspicuous in the ground cherry (fig. 6), while it is not so noticeable in the potato (fig. 7). Near the cotyledonary plate there is a beginning of the centripetal differentiation of the protoxylem and a centrifugal differentiation of the metaxylem.

The behavior of the vascular elements beyond the cotyledonary plate was followed in the petiole and midrib of one of the cotyledons.[1] In the cotyledonary petiole and midrib the protoxylem differentiates adaxially and finally is nearer to the upper epidermis than the metaxylem (figs. 7–9). Simultaneously the metaxylem differentiates abaxially until the endarch condition is fully established (figs. 7–9).

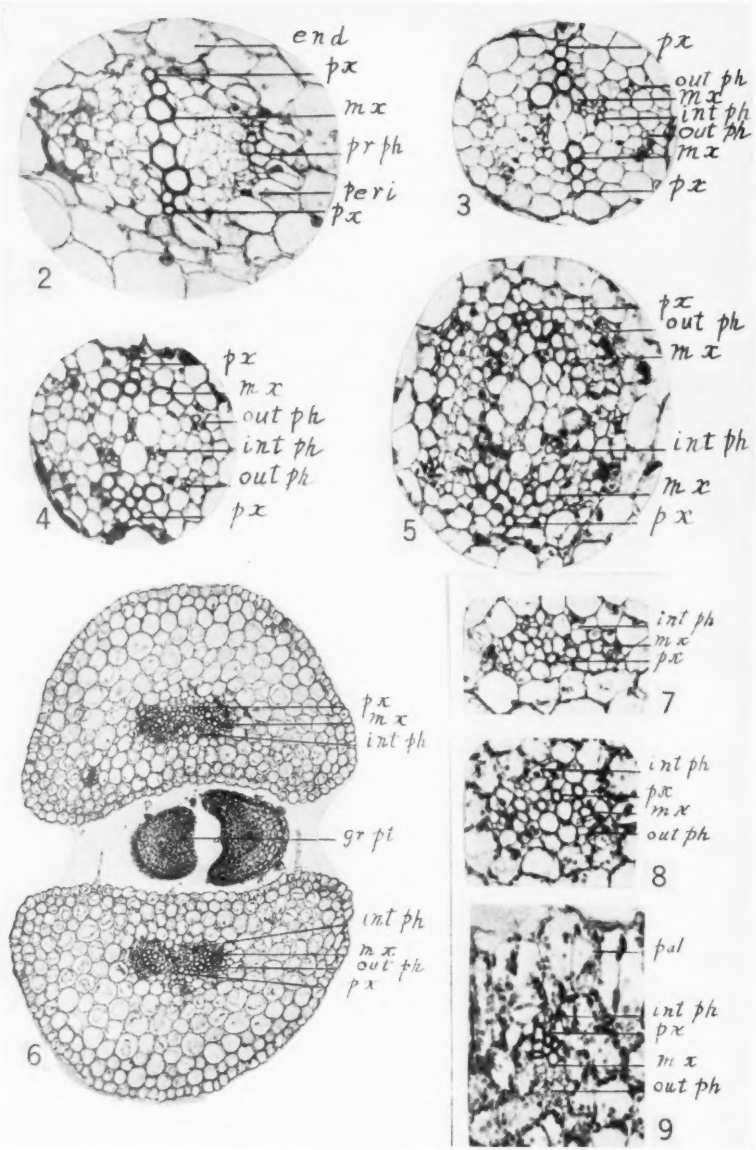
The internal phloem groups are numerous in the cotyledonary petioles (fig. 7). They gradually decrease until there are none remaining in the smaller bundles. In the small bundle shown in figure 9, one small group of inner phloem cells is still noticeable. The outer phloem groups differentiate toward the position originally occupied by the protoxylem point and incline progressively nearer one another until there is complete union of the two groups, thus establishing the bicollateral condition (figs. 6–8).

In the first internode the vascular bundles are completely endarch. Their elements differentiate against those of the hypocotyl slightly below the cotyledonary plate.

Summary

1. Root-stem transition was investigated in *Solanum tuberosum*, *Lycopersicum esculentum*, *Datura tatula*, *Physalis virginiana*, *Petunia acuminata*, and *Solanum pseudo-capsicum*.

[1] The illustrations are oriented so that the adaxial surface of the petiole and lamina are toward the top of the page. See, for example, the position of the vascular elements in the lower cotyledon in figure 6. These will be followed until the endarch condition is established.



FIGS. 2–9.—*On opposite page*

2. The method of transition in each of the plants investigated is the same and agrees with the method in *Solanum melongena*.

3. Illustrations of transition of the potato were used as representative of the other members of the group. The first change from the diarch, radial protostele of the root is a breaking up of the xylem plate and a division of each of the two primary phloem groups into three distinct parts.

4. This is followed by a bifurcation of the metaxylem of the two primary xylem units. These units were not observed to swing outward, one following a left and the other a right curve, as reported by ARTSCHWAGER.

5. The central groups of phloem cells from each side differentiate toward the center of the axis and become the internal phloem. Each of the four remaining groups is gradually inclined in a tangential direction toward the original position of the protoxylem points. This development continues until the bicollateral condition is established.

6. One of the primary xylem units, formed by the breaking of the original diarch xylem plate and consisting of one protoxylem point, its metaxylem, and internal and outer phloem groups, becomes the vascular trace of one cotyledon; the second unit, that of the other.

7. In the cotyledonary petiole and midrib the protoxylem differentiates adaxially and finally is nearer to the upper epidermis than is the metaxylem. Simultaneously the metaxylem differentiates abaxially until the endarch condition is established.

FIGS. 2–9.*—Fig. 2, transverse section of root of potato, showing the diarch, radial protostele; fig. 3, transverse section of hypocotyl showing the two primary xylem units and inward differentiation of two phloem groups; fig. 4, same at higher level, showing bifurcation of metaxylem, the four groups of outer phloem, and several groups of internal phloem; fig. 5, transverse section of hypocotyl 2 mm. below cotyledonary plate showing further bifurcation of metaxylem and the two primary xylem units, each becoming a cotyledonary trace; figs. 2–5, ×500. Fig. 6, transverse section through petioles of cotyledons of the ground cherry, showing position of vascular elements in cotyledons, centripetal differentiation of protoxylem, and centrifugal differentiation of metaxylem. Same situation obtains at this level in the potato plant. ×150. Figs. 7–9, transverse sections through petiole and lamina of cotyledon at successively higher levels; protoxylem differentiates adaxially and metaxylem abaxially until endarch condition is established (adaxial surface of petiole and lamina toward top of page). ×500.

* Abbreviations for all figures: *end*, endodermis; *epi*, epidermis; *gr. pt*, growing point of stem axis; *int.ph*, internal phloem; *mx*, metaxylem; *out.ph*, outer phloem; *peri*, pericycle; *pr.ph*, primary phloem; *px*, protoxylem; *pal*, palisade.

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A NEOGENE SPECIES OF SEQUOIA FROM JAPAN[1]

SEIDO ENDO

(WITH THIRTEEN FIGURES)

Sequoia sempervirens Endlicher **fossilis** Endo.—Cone rather small, about 17 mm. in transverse diameter. Cone scales about 6 in one cycle; distal end of the ovule bearing rhomboidal scale 10 mm. wide and 4 mm. high, with surface transversely grooved and radially wrinkled. Peduncle stout, 3± mm. in diameter. Leaves with decurrent base, sessile, spirally arranged, of two kinds: (1) those on the lateral branchlets, arranged in two lines, having twisted base, linear or lance-shaped, and bluntly mucronate at apex, 10 mm. long, about 2 mm. wide, upper surface slightly furrowed along the midrib; (2) those on the leading and cone-bearing shoots, arranged in several ranks, rather short, not longer than 5 mm., oblong, curved like a horn and pointed (figs. 1–9).

The distichous foliage does not continue uninterruptedly for any considerable distance, but frequently alternates with the imbricated type. Stomata arranged longitudinally and discontinuously on either side of the midrib on the under surface of the leaves; each stoma ringed by four or five narrow subsidiary cells.

LOCALITIES.—1. Middle Umoregi group; a river bed of Hirosegawa just under the Otamaya-bashi, Komega-fukuro, Sendai; figs. 1, 3, 4, 6 (collected by *S. Endo*).

2. Lower Umoregi group; Sanjunin-machi, Sendai (collected by *S. Endo*).

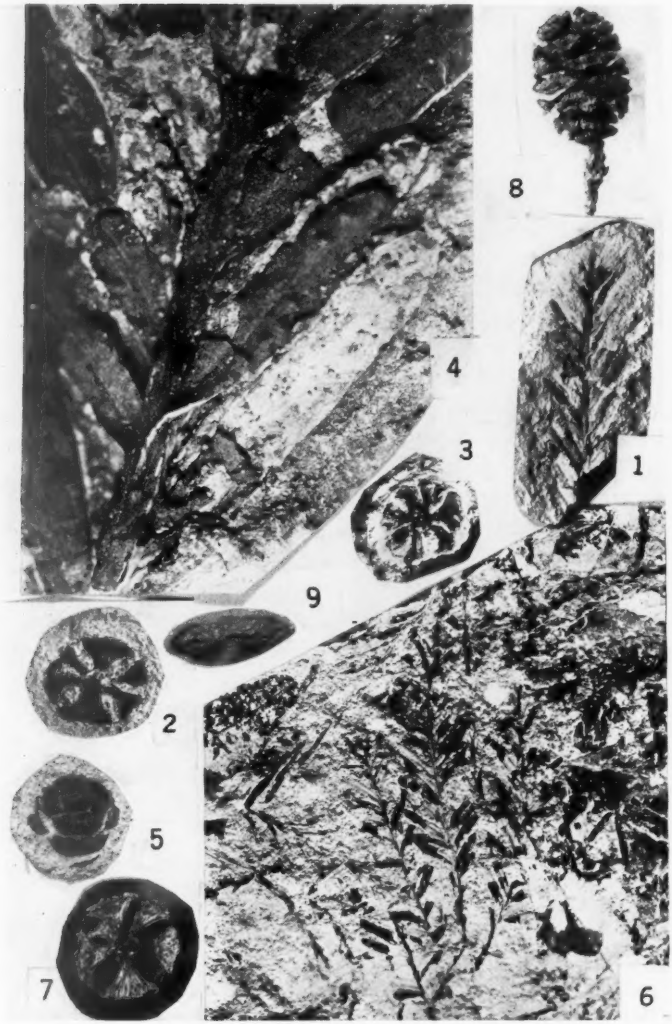
3. Saboyama group; Shiogama-machi near Sendai; figs. 2, 5 (collected by *H. Ozaki*).

4. Sekiya group; Hokine-mura, Shioya-gori, province of Shimotsuke (collected by *H. Niino*).

5. Unknown horizon; Tateyama-shita, Iwayado-machi, Esashi-gori, province of Rikutiu (collected by *H. Yabe* and *F. Saito*).

The present material is identical in the outer features of the cones

[1] Contribution from the Institute of Geology and Palaeontology, Tohoku Imperial University, Sendai.



FIGS. 1–9.—Fossil and living material of *Sequoia sempervirens* Endlicher: figs. 1, 6, examples of foliage; figs. 2, 3, 5, 7, 8, cones; fig. 4, portion enlarged from fig. 1 to show mode of attachment of leaflets to branch; figs. 2, 3, 7, cross-sections of cones showing characteristic cone scales; fig. 5, ornamentation of escutcheons; fig. 9, surface of escutcheon of fig. 5; figs. 7, 8, living material. ×2.

and leaves with *Sequoia sempervirens* Endlicher, found in the western coastal region of the United States in Oregon and northern California (figs. 7, 8). In the fossils, the distichous foliage does not continue uninterruptedly for any considerable distance, but is in frequent alternation with the imbricated type, as already stated. In this respect also it is closely allied to the existing *S. sempervirens*.

The leaves are not so close to the branchlet as in *Sequoia chinensis* (Endo) (1) and *S. disticha* (Heer) (4), and the cones are conspicuously different from *S. chinensis*.

Sequoia tournalii (Brongniart) (4), described by A. G. NATHORST from Shimoshino-kinai-mura, Senboku-gori, province of Ugo, is another closely allied species, although not definitely referable to the present species without cones.

The cones and the leaves just described are found with erect stumps, which are covered by a pumice bed, the so-called "Hirosegawa pumice bed," which is about 4 m. in thickness at this place. It is probable that the forest which produced the present erect stumps was destroyed by this volcanic material. In a basal portion of the volcanic materials of this pumice bed there is a plant-bearing deposit, about 3 cm. thick, with abundant leaves and cones of *Sequoia*. Such being the case, it would seem that these *Sequoia* remains had been detached from the present erect stumps.

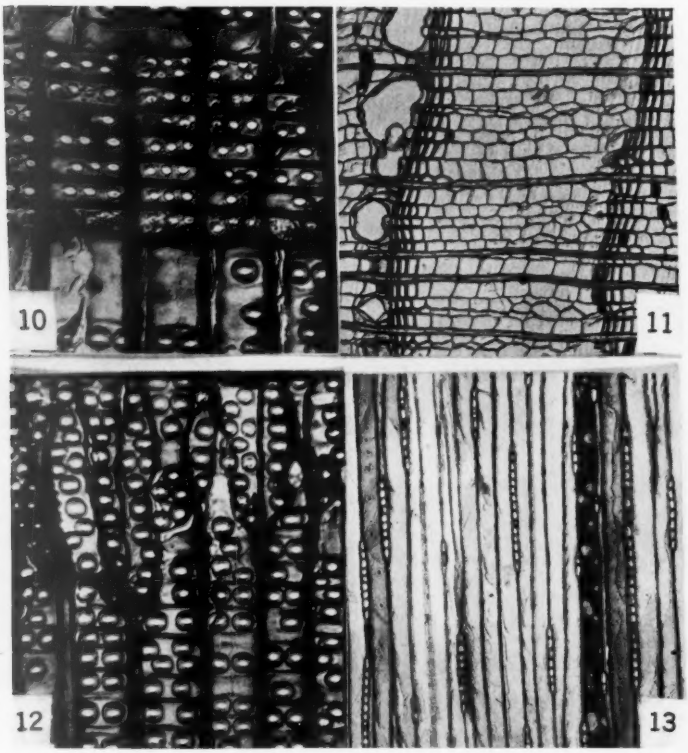
The internal structure of the erect stump was referred by the writer, some years ago, to *Taxodioxylon sequoianum* (Merckl.) Schmalh. erw. Goth. em. (figs. 10–13). This species is similar to *Sequoia sempervirens* in the internal structure, as GOTHAN (2) has stated. Recently TAKAMATSU (6) studied critically the woody structure of the erect stumps at this locality and came to the same conclusion.

GEOLOGICAL AGE.—The three groups cited above from the province of Rikuzen, the Middle Umoregi, the Lower Umoregi, and the Saboyama, are almost certainly Miocene in age, as will be discussed later.

According to SHIMIZU (10) the Cenozoic deposits of the environs of Sendai are divided into groups as follows:

1. Aobayama (Pleistocene); unconformable to the underlying Neogene formations.

2. Upper Umoregi, 10–15 meters; unconformably overlies the next older group at places.
3. Middle Umoregi, 18.5–22.5 m.; unconformable to the underlying group.



FIGS. 10–13.—*Taxodioxylon sequoianum* Gothan, from Middle Umoregi group; a river bed of Hirose-gawa just under the Otamaya-bashi, Komega-fukuro, Sendai: figs. 10, 12, radial longitudinal section of wood; fig. 11, cross-section of wood; ×100; fig. 10, radial longitudinal section of wood showing pits of tracheids; ×350; fig. 13, tangential longitudinal section. ×50.

4. Tatsunokuchi, 30 m.
5. Lower Umoregi, 15.8 m.; this and the upper part of the next or Mitaki group are contemporaneous, although in two different facies of sediments.

6. Mitaki, 20–25 m.; this group shows an apparent gradual transition downward to the Saboyama group.
7. Saboyama, 30 m.
8. Akiu, 115 m.
9. Moniwa, 45 m.

The Tatsunokuchi group is regarded as belonging to the Upper Miocene, the Upper Mizuho (**8**), from molluscan fauna (**9**) and mammalian remains (**3**).

According to NIINO (**5**) the Tertiary deposits near the Shiobara hot-spring district, Shioya-gori, province of Shimotsuke, are divided as follows:

1. Shiobara plant bed, Pleistocene (**7**); unconformable to the underlying Tertiary formations.
2. Sekiya group; unconformable to the next older group.
3. Kanomata-sawa group.
4. Kawaji group, Paleozoic.

The Kanomata-sawa group almost certainly corresponds to the Tatsunokuchi group in age from molluscan fauna (**11**). The other locality of *Sequoia sempervirens* Endlicher **fossilis** is Tateyama-shita of Iwayado-machi in Esashi-gori, province of Rikutiu. This is not yet fully investigated, either stratigraphically or paleontologically.

From the geological occurrences it appears that *Sequoia sempervirens* was widely distributed during the Miocene, but it was absent in Japan in the Middle Pliocene.

The writer wishes to express sincere thanks to Professor H. YABE for his valuable advice and assistance during the present study, and also to Mr. M. TAKAMATSU for assistance with the illustrations.

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A CRETACEOUS SWEET GUM[1]

ROLAND W. BROWN

(WITH ONE FIGURE)

During the latter part of June, 1930, the writer visited a fossil plant locality about 20 miles north of Kemmerer, Wyoming, at the junction of the northeastwardly flowing Everly Creek and Fontanelle Creek which comes down from the north and northwest. At this point the walls of the narrow gorge of Everly Creek and the south cliff facing the bend in Fontanelle Creek display a considerable section of the Upper Cretaceous Aspen shale, which approximates 1000 feet in thickness. The term shale as applied to the Aspen of this section includes strata of black and gray shales, clay, thin coals, bentonite, gray sandstone, and light-colored tuff, all dipping 35° westward and striking almost due north and south. The gray shales weather into long rounded hills with a distinctive greenish gray or silver gray appearance. The fossil plants are imbedded in the uppermost 125 feet of the formation, in a thin stratum of bluish gray, hard mudstone which is very brittle and fractures conchoidally. The plants are disposed at all angles through the matrix, making it somewhat difficult to get entire specimens. They are very well preserved and stand out black against the bluish gray background.

Of the fourteen species of plants in the collection,[2] the most attractive and suggestive form is one that I have called *Liquidambar fontanella*. Figure 1 illustrates the largest fragment of this species, but there were also in the collection numerous other fragments which permit the reconstruction of the entire leaf as given. So far as the remains permit a conclusion, all these leaves were 3-lobed, whereas the range of variation in modern sweet gums includes 3 to 7-lobed leaves, with the 5-lobed leaf the usual and most common form.

The breadth of the *Liquidambar fontanella* leaf from tip to tip of

[1] Published with the permission of the Director of the United States Geological Survey.

[2] BROWN, R. W., Fossil plants from the Aspen shales of southwestern Wyoming. U.S. Nat. Museum Proc. (In press.)

the lower lobes is 13 cm., and the length from the top of the petiole to the tip of the middle lobe is 8 cm. The lobes are 1 cm. wide near the base and become narrowly attenuate to their tips. The margin is finely crenate-serrate. The base is slightly cordate. The total length of the petiole is unknown. Three strong veins arising from the top of the petiole comprise the primary palmate venation, the laterals diverging at an angle of 60° from the middle vein. Eight to ten pairs of secondaries, appropriately spaced, branch off from the

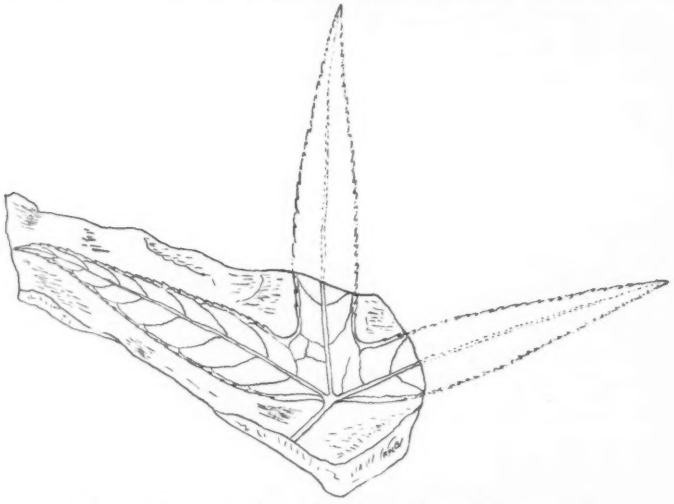

FIG. 1.—Reconstruction of *Liquidambar fontanella* Brown. $\times\frac{2}{3}$

primaries at approximately 60°, loop upward near the margin, and connect with the secondaries above. No finer details are discernible with a hand lens, and since no part of the epidermal or cuticular substance of the leaf remains, no microscopic studies can be applied. In general the characters of this leaf compare with none better than with those of the modern sweet gum leaf. The fossils differ chiefly in having unusually long lobes and a more restricted area of the basal region. From an esthetic point of view, the tree which bore these delicately graceful leaves was undoubtedly a striking object in the Cretaceous landscape along the stream courses and moist low country of southwestern Wyoming.

No remains of the characteristic *Liquidambar* fruits, called sweet gum balls, were found with the fossil leaves. This lack is regrettable, and it suggests the propriety of further search in the Aspen shales for these distinctive fruits, as well as for petrified wood and flowers, which would tend to confirm the determination of the leaves. Perhaps the identification as it now stands may be regarded by some as merely conjectural. VELENOVSKY (**6**), for example, has described and figured some 3-lobed leaves, called *Aralia formosa* Heer, from the Cretaceous of Bohemia. These, while superficially resembling *Liquidambar fontanella*, differ markedly in venation and in having a wedge-shaped rather than a cordate base.

A review (**1**) of the fossil sweet gums shows that some 20 Eurasian and 13 American species have been described, mostly from Tertiary and recent deposits. These remains include unmistakable flowers, fruits, and leaves. The earliest sweet gum was reported by HEER (**3**) from the Upper Cretaceous Dakota sandstone of Kansas. This leaf, called *Liquidambar obtusilobatus* (Heer) Hollick (**5**), as well as several other Cretaceous species, had the general aspect of a sweet gum leaf except that the margin was entire, a character which is now thought to ally it with *Sterculia* or *Aralia* rather than with *Liquidambar*. In view of these discredited Cretaceous species, the form now under discussion, *Liquidambar fontanella* Brown, becomes the earliest known sweet gum, or at least the earliest with leaves having crenate-serrate margins.

Whether *Liquidambar* originated from angiospermous ancestors with entire-margined leaves is a question. So far as I know, the study of juvenile, atavistic, or teratologic leaves of the living sweet gums does not reveal any with entire margins. HOLM (**4**), in a study of the leaf variations of the eastern American *L. styraciflua* Linné, showed that the leaves, shade leaves particularly, on seedlings were frequently entire, ovate, sometimes slightly lobed, but in all cases with toothed margins.

Sweet gum remains are well known from the American Cenozoic deposits. *L. callarche* Cockerell is present in the oil shales of the middle Eocene Green River formation, occurring in the contiguous corners of Colorado, Wyoming, and Utah. *L. californicum* Lesquereux from the auriferous gravels of California; *L. convexum* Cockerell

from the lake beds of Florissant, Colorado; and *L. pachyphyllum* Knowlton from the Mascall formation of the John Day Basin, Oregon, are recognized Miocene species. From the Pleistocene deposits of the Pamlico ("Chowan") formation near Seven Springs, Wayne County, North Carolina, BERRY (2) has recorded the fruits and leaves of the living *L. styraciflua* Linné.

The most familiar European fossil species is *L. europaeum* Al. Braun, from various Miocene localities, but principally from the celebrated lake beds at Oeningen, in Baden, Germany. There were, however, sweet gums in the Eocene and also in the later Tertiary of Europe, eastern Asia, and Japan. In short, the distribution of the genus *Liquidambar* was holarctic during Tertiary time, but during the Pleistocene glaciation many species were exterminated and the present restricted distribution of the four living species was brought about: one in central and southeastern China and Formosa; one in Asia Minor; one in southeastern United States and Mexico; and one in the uplands of Guatemala.

In what region of the world the genus *Liquidambar* became differentiated as such from its ancestors and relatives; how its distinct species were evolved; and by what routes and for what causes the species distributed are questions inviting speculation, but to which there appear to be no definite answers at the present writing. No small part of the difficulty here lies in the fact that there is no satisfactory method for distinguishing the fossil liquidambars on the basis of foliar remains alone. Distinction of species is largely by inference, which suggests that apparently similar leaves found in strata differing in age by a million years would most likely be recognized as distinct species if the trees on which they grew could now be placed side by side for comparison in their entirety.

Summary

1. *Liquidambar fontanella* Brown, together with thirteen other fossil plants, was obtained in a collection from the Upper Cretaceous Aspen shale in southwestern Wyoming.

2. The foliar characters of *L. fontanella* are almost identical with those of the living sweet gums, and justify its tentative assignment to the genus *Liquidambar*.

3. The genus *Liquidambar* achieved a holarctic distribution in Tertiary times, but during the Pleistocene glaciation was reduced to four species with restricted distribution.

4. The relation between *L. fontanella* and all other sweet gums, fossil and living, is conjectural; but it seems that *L. fontanella* is the earliest recognizable sweet gum which resembles living species.

UNITED STATES GEOLOGICAL SURVEY
WASHINGTON, D.C.

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6. VELENOVSKY, JOSEF, Die Flora der böhmischen Kreideformation. p. 21. pl. 5 (3), fig. 2; pl. 6 (4), fig. 7; pl. 7 (5), figs. 2, 3, 4. 1882.

"GEO-GROWTH" REACTION OF ROOTS OF LUPINUS

A. E. NAVEZ

(WITH ONE FIGURE)

A recent paper by KEEBLE, NELSON, and SNOW[1] has raised again the question of the existence of a geo-growth reaction in roots. Working with *Zea mays*, they reported that roots placed horizontally increased their rate of growth by as much as 50 per cent when measurements were made over periods of about 6 hours. They stated also that roots of *Pisum*, although not giving as clear-cut results, showed the same definite reaction. As the writer could not find such a geo-growth reaction in coleoptiles of *Avena*,[2] it was thought well to check the observations recorded by the British investigators, especially in view of the fact that tropistic movements of roots of *Lupinus* had been studied without such a reaction having been noticed.

During the past summer, the time-elongation curve was followed with a great number of roots of seedlings of *Lupinus albus* grown in moist sawdust at 22°–23° C. Measurements were made at first by means of a finely divided ruler; later by measuring with a curvimeter the enlarged photographical image of the roots kept undisturbed in their growing chamber. This latter process of measurement is simple with the aid of a camera with double extension and an objective which can be demounted, and whose back half can be used to give an enlargement of about twice that of the complete optical system. Outlines on light tracing paper can be taken very rapidly and with considerable precision.

Elongation of the normal, vertical root was found to proceed at constant rate between 30 and 90 hours after the start of germination. On the average, the roots during this time grew from 10 mm. to 50 mm. in length. The seedlings were grown in very moist sawdust and kept on this medium between periods of measurements, at which brief times they were transferred to a covered glass cell containing about 1 cm. of water, and lined almost completely, except

[1] KEEBLE, E., NELSON, M. G., and SNOW, R., Proc. Roy. Soc. B. **108**:360. 1931.

[2] NAVEZ, A. E., and ROBINSON, T. W., Jour. Gen. Physiol. **16**:133. 1932.

for two small openings at the front and back, with water-soaked blotting paper. Occasionally seedlings were maintained for longer durations in the observation cell. It should be noted that under all circumstances the roots were fully turgid, and could be kept growing at constant rate for periods up to 8 and 10 hours in a saturated atmosphere, when handled as here described. Care was taken to remove any adhering particles of sawdust and the excess water by means of a fine dry brush.

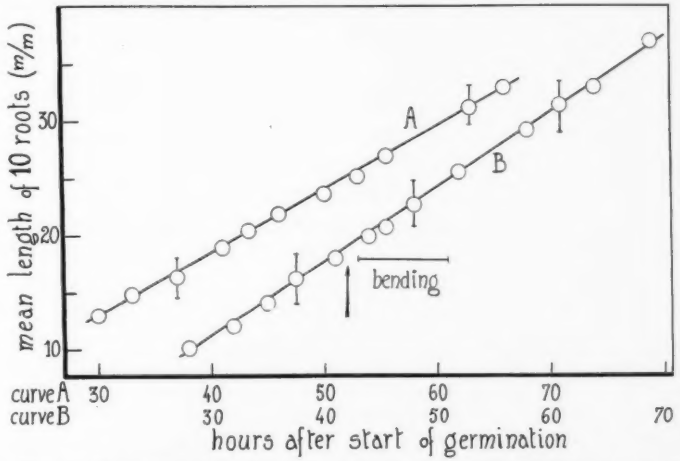

FIG. 1.—Time-mean elongation curve of roots of: *A*, 10 seedlings kept vertically throughout the experiment; *B*, 10 seedlings kept vertically, then turned horizontally (moment of rotation marked by arrow). Both series of observation points are best fitted by straight lines. For both curves the length of the short verticals above and below a few of the observation points indicates the extremes among the lengths averaged. The difference in the slopes of curves *A* and *B* results from the different temperatures (20.3° and 22° C.) at which they were obtained.

Under these conditions, as is seen in figure 1, the statement of KEEBLE and his co-workers cannot be confirmed: no geo-growth reaction of the root was shown by these seedlings. The behavior of the roots they observed must be accounted for in some other way. Of course one could think that the difference in material might explain the difference in results. At the time of the present experiments, no material of *Zea mays* was available. More recently an experiment was performed with *Z. mays*, however, involving 48 seeds, and the phenomenon was not detectable.

There is the possibility of error resulting from the technique used by KEEBLE and his co-workers. In brief, their method was as follows: an outline of the root was made on which a thread was laid, along the middle line of the drawing; the length of the root was then known by transfer of the thread to a graduated scale. The method of expressing the measurements in terms of percentage of growth of the straight root may not seem conducive to a high degree of accuracy; but, disregarding these possible errors, the differences recorded are of sufficient magnitude that they cannot be attributed entirely to the technique of measurement and to the method of expressing these measurements.

The cause of divergence, therefore, must probably reside in the difference of experimental technique. It had been noted that seedlings in the vertical position showed a reduced rate of growth when a drop of water was hanging at the tip of the root. It was easy to show that this drop of water, even when small, would retard elongation of the root, and that when it was removed normal elongation was resumed.

In the case of a root placed horizontally, the presence of a drop of water at the very tip is almost impossible; it would spread rapidly in a film over the apical region with a definite excess on the lower side of the horizontal root. This, of course, would make the conditions of growth different for the two positions, and results such as those reported by KEEBLE and his collaborators could be expected. In cases in which the roots have the excess water removed, no difference in the rate of elongation can be found.

While this paper was being prepared for publication, CHOLODNY[3] published a series of observations on the growth of roots of *Zea mays* which are in perfect agreement with the views here expressed.

It would seem logical to ascribe to a technical imperfection the results reported by KEEBLE, NELSON, and SNOW, and to maintain until further adequate study that roots of *Lupinus albus* and *Zea*, as well as coleoptiles of *Avena*, do not show increased rate of elongation during geotropic response.

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[3] CHOLODNY, N., Arch. Wiss. Bot. (Planta) **17**:794. 1932.

BRIEFER ARTICLES

PARTIAL PHYLLODY OF YUCCA ELATA

(WITH ONE FIGURE)

Observations on phyllody in *Yucca elata* Engelm., previously reported from the Jornada Experimental Range in southern New Mexico, dealt with specimens in which differentiation of flowers failed completely.[1] All of the cases found from 1926 to 1931 were of this type. In July, 1932, however, three specimens of partial phyllody were noticed. These specimens of partial phyllody, two of which are shown in figure 1, were produced in 1931, probably during the early summer. When they were found a year later, the panicles were dry, the capsules open, and most of the seeds were dispersed. Even though the stalks were a year old, they were sufficiently well preserved to demonstrate this unusual feature for *Y. elata*.

The usual inflorescence of *Y. elata* is a much branched panicle with perfect flowers. It has been reported previously[2] that the number of blossoms on different flower stalks may vary from 75 to sometimes more than 200, but the number of flowers which produce mature capsules is low, rarely as high as 30 per cent. Observation of the flower stalks shown in figure 1 revealed that flowers and mature capsules were produced on the lower branches of the panicles, but flowers failed to differentiate on the upper portions, thus producing the condition of partial phyllody. The smaller panicle produced nine normal branches, supporting approximately 50 flowers; five branches showing transition between typical growth and phyllody; 14 leaf clusters on the main axis and a dense terminal group of leaf clusters. Five of the flowers produced mature capsules. The larger panicle (fig. 1) produced 25 normal branches, with approximately 100 flowers, six transitional branches, nine branches in condition of phyllody, and the terminal leaf cluster. Eleven mature capsules were produced on this specimen.

It is impossible to determine the cause of the condition observed from the scanty material available. Both of the specimens were inhabited by the carpenter bee, *Xylocopa californica*, but this insect does not enter

[1] CAMPBELL, R. S., A case of phyllody in *Yucca elata*. BOT. GAZ. **88**:109–110. 1929.

[2] CAMPBELL, R. S., and KELLER, J. G., Growth and reproduction of *Yucca elata*. Ecology **13**:364–374. 1932.

Yucca flower stalks until after the flowers are fully developed and it is by no means confined to panicles in a condition of phyllody. The specimens shown in figure 1 were taken from stalks with a connecting underground stem.

FIG. 1.—Two flower stalks of *Yucca elata*, showing partial phyllody

Although many observations have been made, no cases of phyllody have been observed in other species of *Yucca* found in southern New Mexico, *Y. baccata*, *Y. glauca*, and *Y. macrocarpa*.—R. S. CAMPBELL and J. G. KELLER, *Southwestern Forest and Range Experiment Station, U.S. Forest Service, Las Cruces, New Mexico.*

AN ADAPTER FOR PHOTOMICROGRAPHY WITH BOX CAMERAS

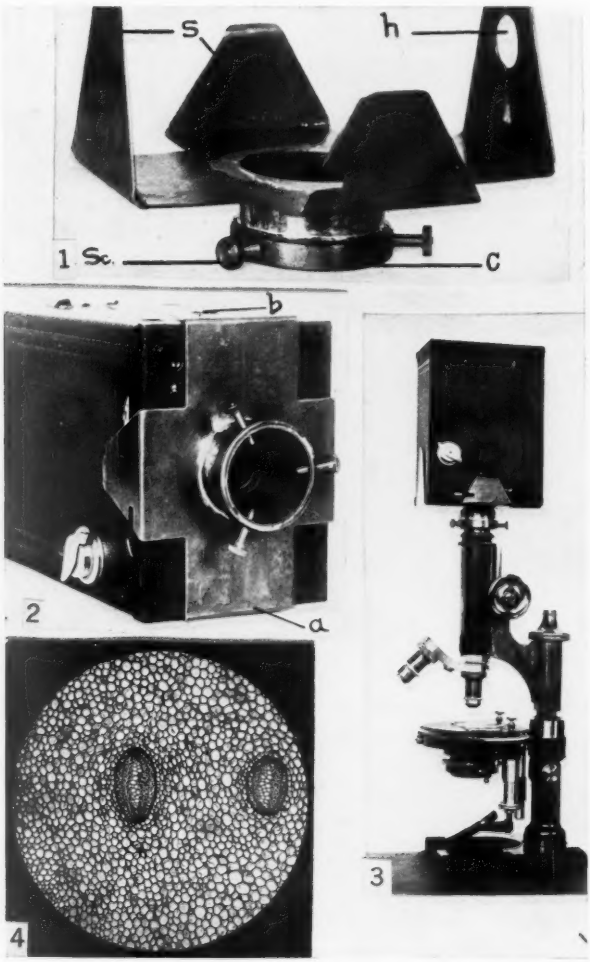
(WITH FOUR FIGURES)

The use of such a cheap box camera as a Kodak is perhaps rare in the field of photomicrography. With such a camera, however, photographs can be taken with great ease and precision, and with considerable economy of time. The process is simple, consisting in taking the picture of a focused object by fixing the opening of the camera directly on the eye-piece of the microscope. With a time exposure, however, it is difficult to hold the camera steadily for the time required. To remove this difficulty, a simple piece of apparatus has been devised which works very satisfactorily.

The adapter is made of a brass sheet about 1 mm. thick. It consists of a collar (length 20 mm., inner diameter 32 mm.) soldered to the central hole (diameter 34 mm.) of a brass plate cut in the form of a cross (figs. 1, 2). The collar is provided with three screws. A portion of each limb of the brass plate is bent at right angles to its central part. Provision is made, by proper trimmings and holes in the bent limbs, for the operation of the shutter, time-exposure bar, etc., without interference when the adapter is fixed on to the camera.

The object to be photomicrographed is first correctly focused under critical illumination of the microscope. The adapter is then slipped over the face of a loaded camera, so that it grips the four sides of the box rather firmly. The time exposure bar is pulled out, the apparatus adjusted to bring the opening of the camera flush with the surface of the eye-piece of the microscope, and the collar screws tightened (fig. 3).

The time exposure will vary, of course, with the intensity of light and the kind of plate or film used. With an ordinary film or plate, and with the iris-diaphragm of the microscope open full, an exposure of about two seconds suffices in the ordinary daylight of the laboratory. A specimen of a photomicrograph (a transverse section of the rhizome of *Leucostegia hymenophylla*) taken with this arrangement is reproduced in figure 4. The depth of focus obtained is even throughout the field and no blurring is noticeable toward the periphery, as is usually the case in most photomicrographs.—T. C. N. SINGH, *Botany Department, Ravenshaw College, Cuttack, India.*



FIGS. 1–4.—Fig. 1, the adapter: *c*, collar; *s*, vertical sides; *sc*, screw; *h*, hole for handle button of camera. Fig. 2, camera with adapter in place. Fig. 3, camera adjusted to microscope. Fig. 4, photomicrograph of transverse section of rhizome of *Leucostegia hymenophylla* taken with the arrangement as shown in fig. 3.

CURRENT LITERATURE

English edition of Braun-Blanquet's Pflanzensoziologie

A recent author has characterized ecology as "the science of communities." Whether plant ecologists generally will accept this restricted definition of their field is extremely doubtful; among English speaking botanists, at any rate, long usage has firmly implanted the idea of plant ecology as the study both of plant communities and of individual plants in their relation to environment. Be this as it may, there can be no question that the study of plant communities, or plant sociology, if not the most important, constitutes at any rate the most unique phase of plant ecology, the phase in which it is most distinct from all other fields of plant science. The present volume[1] is one of the very few modern textbooks which deals specifically with the subject of plant sociology.

As described by the author, the field of plant sociology embraces all knowledge of vegetation in the widest sense; it includes all phenomena which touch upon the life of plants growing in social units. It has to do with the investigation of plant communities with reference to their organization, structure, and composition, their dependence upon one another and upon environment (synecology), their developmental relations (syngenetics), their geographical distribution (synchorology), and their classification (systematics). These diverse points of view form the basis of treatment in the text, which is divided into five principal sections, one for each of these heads, in addition to an introductory section on social life among plants. A review of the original German edition has already appeared in these pages.[2] It should be emphasized again, however, that the book is of special interest by reason of the attention it directs to various concepts and phases of plant community study which have been highly developed in continental Europe while largely neglected in this country; in particular, to the structural features of the community and the methods by which these features can be studied, evaluated, and coordinated. In this respect the book is outstanding in its field. Of special note also is the clear and extended discussion of the habitat factors, which are classified from a distinctly ecological point of view into six groups, namely, climatic factors, edaphic or soil factors, soil organisms, soil types, orographic factors, and biotic factors.

In the preparation of the English edition the text has been thoroughly revised, and in addition various topics have been introduced which did not appear in the original German edition. In its present form, thanks to the combined

[1] BRAUN-BLANQUET, J., Plant sociology. Translated, revised, and edited by FULLER, GEORGE D., and CONARD, HENRY S. pp. xviii+439. figs. 180. Bibliography. McGraw-Hill Book Co. New York. 1932.

[2] BOT. GAZ. **88**:451, 452. 1929.

skill of author and editors, the book represents a comprehensive, well-balanced, and scholarly exposition of the science of plant communities, constituting a notable achievement in the progress of plant ecology.—G. E. NICHOLS.

Handbook of soil science

Beginning in 1929, there have appeared at intervals the various volumes of the handbook of soil science.[3] This work is edited by E. BLANCK of the University of Göttingen. He is also the author of a number of sections and has been extensively aided by other German soil scientists.

In nature and content, the volumes may be divided into two groups. The first seven are concerned with general features of soil science. The processes of soil formation and the various factors involved are discussed in detail. The various soil types are described and their geographical distribution given. There is a full discussion of the physical, chemical, and biological characteristics of the soil. The last three volumes deal with special or applied features of soil science. In these volumes are discussed such subjects as: methods of determining the fertility of the soil; principles of soil tillage; manuring the soil; and methods of modifying the activity of soil microorganisms. The last volume discusses the technical uses of the soil.

Taken as a whole, the work is an excellent and comprehensive treatise of the entire field of soil science, both pure and applied. The ten volumes comprise a total of nearly 5000 pages and there are over 600 illustrations. The handbook should be of great help to all interested in this subject.—S. V. EATON.

Environmental factors

Realizing the impossibility of keeping familiar with the increasing multitude of scientific publications, the ecologists and pathologists will welcome a bibliography.[4] This pamphlet includes 3689 citations arranged alphabetically, with three classified indexes of environmental factors, host-disease injury, and hosts respectively. Its usefulness is unquestionable.—G. D. FULLER.

Shrubs of Indiana

As a companion volume to the *Trees of Indiana* recently noted in this journal,[5] DEAM has now written a similar volume on the shrubs.[6] Its most valuable features are the distribution maps, the excellent illustrations, and the ecological notes. The arrangement of the material, the details of peculiar distribution, the quality of the illustrations, and the excellence of the typographical work all make it a model that may well be followed.—G. D. FULLER.

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[3] BLANCK, E., Editor, Handbuch der Bodenlehre. Vols. I–X. Hirschwaldsche Buchhandlung, Berlin. 1929–1932.

[4] WILSON, J. D., Environmental factors in relation to plant disease and injury: A bibliography. Ohio Agric. Exp. Station Technical Series. Bull. 9. pp. 203. Wooster, Ohio. $1.00. 1932.

[5] BOT. GAZ. **93**:492–493. 1932.

[6] DEAM, CHAS. C., Shrubs of Indiana. Dept. of Conservation, State of Indiana, Publ. 44. 380 pp. 155 maps. 153 pl. 1932.